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Optimising therapeutic strategies for chronic rhinosinusitis

Vaidyanathan, Sriram

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Sriram Vaidyanathan

2014

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Optimising therapeutic strategies for chronic rhinosinusitis

Mr Sriram Vaidyanathan

MBBS, MRCS, DOHNS, FRCR

Doctor of Medicine (MD)

University of Dundee

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Acknowledgements, Declaration,
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I would like to dedicate this MD thesis to my eternal guide and mentor in life Dr Daisaku Ikeda, who has never left my side.

Declaration

I, SRIRAM VAIDYANATHAN, am the sole author of this thesis. The clinical research herein was carried out in the Asthma and Allergy Research Group, The Department of Cardiovascular and Lung Biology, University of Dundee, Ninewells Hospital, under the clinical and educational supervision of Professor Brian Lipworth. I worked with other research fellows in the department - Dr Peter Williamson, Dr Arun Nair and Dr Karine Clearie during this period. Clinical research technicians within the department ran the clinical trials. I was responsible for supervision of the studies in this thesis with cover from the other research fellows during periods of absence.

The second study was designed by Dr Martyn Barnes and Professor Brian Lipworth 3 years prior to my starting in the department. From January 2007 I was responsible for dealing with the day-to-day management of this study and boosting recruitment. I took over as principal investigator from Dr A Nair in February 2008. I successfully acquired an NHS Tayside P & N Small Grants Scheme grant (R04099 E599) towards this study. I was also responsible for all the data analysis, organizing further laboratory analysis and writing-up of this section of the trial. The other grants towards this study were from the Chief Scientist Office, Scotland (CZG/1/123) and the Anonymous Trust Grant Scheme, University of Dundee (GEN/231/51/BMcK/JCL). None of the funding bodies were involved in the study conceptualization, design, analysis or write-up.

All the other studies included in this thesis are solely my work from conceptualization, design, regulatory approval, recruitment, data analysis, peer-reviewed publication and the final presentation in this manuscript. Funding for these

studies was primarily obtained as unrestricted educational grants from the Asthma and Allergy Research Group, University of Dundee. I am responsible for the integrity of the data and the analysis thereof. I have consulted the references or abstracts cited. This work has not been accepted nor submitted for a research degree elsewhere.

Publications arising from this thesis

1. Vaidyanathan S, Williamson P, Anderson K, Lipworth B. Effect of systemic steroids on humming nasal nitric oxide in chronic rhinosinusitis with nasal polyposis. *Annals of Allergy Asthma and Immunology*. 2010 Dec;105(6):412-7.
2. Vaidyanathan S, Barnes M, Williamson P, Hopkinson P, Donnan PT, Lipworth B. Treatment of chronic rhinosinusitis with nasal polyposis with oral steroids followed by topical steroids: a randomized trial. *Annals of Internal Medicine*. 2011 Mar 1;154(5):293-302.
3. Vaidyanathan S, Williamson P, Lipworth B. Is a positive nasal lysine-aspirin challenge test associated with a more severe phenotype of chronic rhinosinusitis and asthma? *American Journal of Rhinology*. 26, 1-5, 2012. Doi 10.2500/ ajra.2012.26.3767.
4. Vaidyanathan S, Williamson P, Clearie K, Khan F, Lipworth B. Fluticasone reverses oxymetazoline-induced tachyphylaxis of response and rebound congestion. *Am J Respir Crit Care Med*. 2010 Jul 1;182(1):19-24.
5. Vaidyanathan S, Williamson P, Lipworth B. Comparative evaluation of nasal blood flow and airflow in the decongestant response to oxymetazoline. *Ann Allergy Asthma Immunol*. 2012 Feb;108(2):77-80.

Oral and Poster Presentations

1. Fluticasone Reverses Oxymetazoline Induced Tachyphylaxis and Rebound Congestion via Alpha-1 receptor subtype in nasal mucosa. Association of Physicians Great Britain and Ireland Annual Meeting 2010. Dundee. Poster.
2. Immunological and molecular factors influencing steroid responsiveness in nasal polyposis. Royal Society of Medicine. October 2009. Podium
3. Role of medical polypectomy in chronic rhinosinusitis with nasal polyposis. Vaidyanathan S, Barnes ML, Gardiner Q, White P, Lipworth BJ. Otolaryngological research society, April 2009, Nottingham. (Podium)
4. To assess if positive nasal lysine-aspirin challenge is a predictor of a more severe phenotype of chronic rhinosinusitis or asthma. S Vaidyanathan, P Williamson, K Clearie, BLipworth. Scottish Thoracic Society, 2009 Spring Meeting. Perth. (Podium). Best Presentation, Methven Prize.
5. To assess the effects of combined nasal and inhaled corticosteroid in patients with asthma and persistent allergic rhinitis. A Nair, S Vaidyanathan, KClearie, P Williamson, B Lipworth. Scottish Thoracic Society, 2009 Spring Meeting. Perth. (Podium).
6. Factors influencing steroid responsiveness in nasal polyposis— role of medical polypectomy. Vaidyanathan S, Barnes ML, Garnider Q, White P, Lipworth BJ. Scottish Otolaryngology Society, 2008 Winter meeting. Aberdeen. (Podium)—Best Research Presentation.
7. Factors affecting treatment response in patients with nasal polyposis. Vaidyanathan S, Barnes ML, Garnider Q, White P, Lipworth BJ. British Thoracic Society, 2008 Winter meeting. London. (Podium)

8. Influence of aspirin sensitivity, asthma and atopy on steroid response in nasal polyposis. Vaidyanathan S, Williamson P, Lipworth BJ. Scottish Thoracic Society, 2008 Winter meeting. Podium. Best Presentation. Methven Prize.

List of Abbreviations

AIR	Aspirin intolerant rhinosinusitis and asthma complex
AR	Allergic rhinosinusitis
ARS	Acute rhinosinusitis
ARIA	Allergic rhinitis and its impact on asthma 2008
CLARI	Clarithromycin
CRS	Chronic rhinosinusitis
CRSwNP	Chronic rhinosinusitis with nasal polyposis
CRSSNP	Chronic rhinosinusitis without nasal polyposis
EPOS	European position paper on rhinosinusitis 2012
FESS	Functional endoscopic sinus surgery
FP	Fluticasone propionate
GA2LEN	Global Allergy and Asthma European Network
INCS	Intranasal corticosteroids
L-ASA	Lysine - Acetyl Salicylic Acid
LDF	Laser Doppler Flowmetry
LTRA	Leukotriene receptor antagonist
NAR	Nasal airways resistance
PNIF	Peak nasal inspiratory flow
QoL	Quality of life
RSDI	Rhinosinusitis disability index
RSOM	Rhinosinusitis outcome measure 31
SE	Staphylococcal enterotoxins
SEA	Staphylococcal enterotoxin type-A
SEB	Staphylococcal enterotoxin type-B
SNOT-20	Sinonasal outcomes test -20 point scale
SNOT-22	Sinonasal outcomes test -22 point scale
TSST-1	Toxic Shock Syndrome Toxin -1

Summary Statement

The aim of this thesis is to evaluate and optimise current pharmacotherapeutic options in rhinosinusitis. There is often a marked variation in treatment response in those afflicted with chronic rhinosinusitis, both within and between patients, attributable in part to different disease phenotypes/endotypes, poor awareness of treatment optimization options, and trivialization of symptoms by patients and physicians. Characteristically, these factors contribute to a typical remitting and relapsing disease course. The objectives of this work are to improve the therapeutic index and reach of commonly used medications by boosting efficacy whilst reducing concomitant side effects.

The third chapter explores the use of initial oral steroids in patients with chronic rhinosinusitis and nasal polyposis, focusing on the role of the ostiomeatal complex in the perpetuation of disease symptoms. Often a short course of oral steroids is used in patients with moderate to severe disease to achieve initial control before maintenance with intranasal steroids. This is termed as a ‘medical polypectomy’ and anecdotally is commonly used in patients with chronic rhinosinusitis with nasal polyposis (CRSwNP). However, the evidence for its efficacy is tenuous and there are no data to evaluate if it indeed re-establishes ostiomeatal sinus complex drainage which is a *condicio sine qua non* of ensuring long-term symptom resolution. Further, it is known that monotherapy with nasal steroids may result in loss of symptom control. We have therefore in a double-blind placebo controlled trial (Chapter 4) evaluated the effect of this initial induction with oral steroids on subsequent sequential intranasal therapy. Perhaps, however, more crucially we have for the first time comprehensively addressed the safety of both oral and topical steroids in patients with CRSwNP who have other concomitant steroid-dependent illnesses like

asthma and COPD. A particularly refractory subset of those with CRSwNP also have aspirin intolerance and asthma. While recent guidelines have recommended more aspirin challenge testing in these patients, it is unclear what the significance of a positive test is in the absence of overt clinical symptoms or in patients with only moderate disease. This is addressed in Chapter 5, as this significant phenotype of aspirin intolerant rhinosinusitis need close monitoring, dose optimization, polytherapy, and in selected cases may be suitable for aspirin desensitization. Penultimately, we evaluate in a double-blind placebo controlled trial (Chapter 6) the tachyphylaxis and rebound congestion that blights the medium to long-term use of sympathomimetic nasal decongestant sprays like oxymetazoline and if this can be reversed by the concomitant use of nasal steroids. We also characterized nasal blood flow as an outcome to evaluate in these patients and its relation to other rhinological outcome measures (Chapter 7).

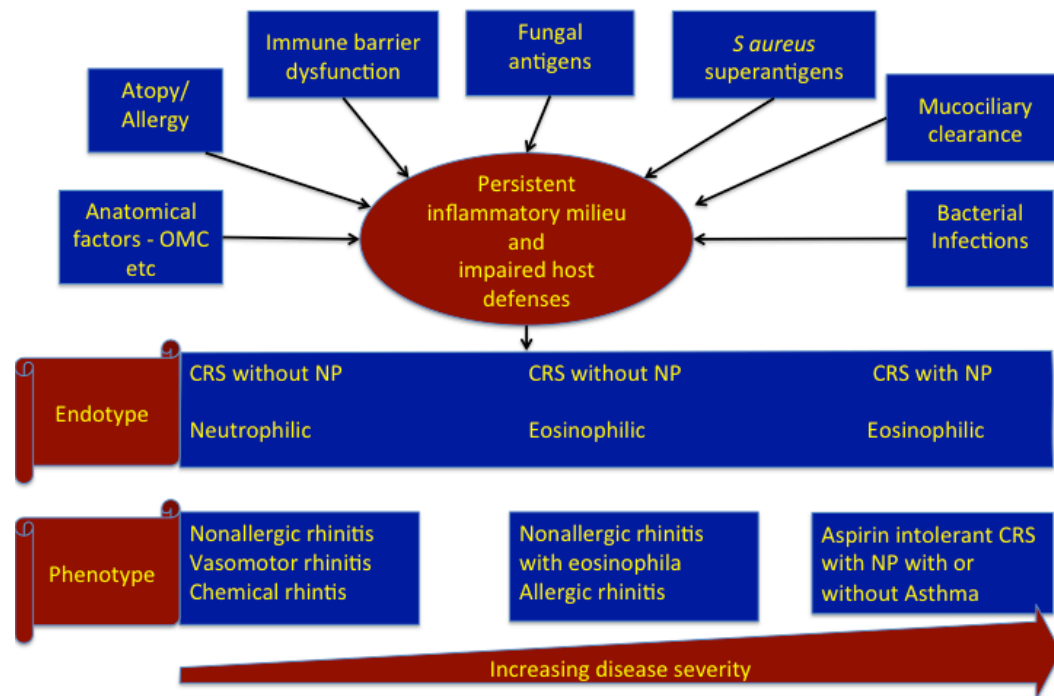
Chapter 1: Introduction and Literature Review

DEFINITIONS

Rhinitis is an umbrella term for a heterogeneous group of disorders, with diverse phenotypes and disease courses, but commonly defined as having inflammation of the nasal mucosa. This inflammation may in turn be influenced by factors such as infections, allergies, chemicals, drugs and hormones. Rhinitis is frequently associated with a variety of debilitating symptoms including rhinorrhoea, postnasal discharge, blockage, sneezing, itching, and hyposmia. Since rhinitis and sinusitis usually contemporaneously manifest themselves in an afflicted individual, the term ‘rhinitis’ has now evolved into the more encompassing ‘rhinosinusitis’. Indeed, most current guidelines now refer to this conglomerate of disorders as rhinosinusitis(1, 2). Among chronic rhinosinusitis (CRS), defined as having symptoms for more than 8-12 weeks, allergic rhinitis (rhinosinusitis) represents the largest disease subtype amongst these disorders and is clearly the best studied. There seems to be an unintended dichotomy within the literature with allergic rhinosinusitis (AR) related research existing almost in parallel with studies on CRS. On one level, this perhaps reflects the fact that rhinosinusitis presents itself to a variety of practitioners such as otolaryngologists, allergologists, respiratory physicians, immunologists, and family physicians among others. On the other hand, this underscores the lack of understanding on how AR, a predominantly IgE mediated process melds into CRS and the delineation of the journey from an initial insult to subsequent tissue change and finally disease perpetuation. The currently surmised role of allergy in CRS will be looked at a bit later. Briefly, there is sparse evidence to support the role of allergy in CRS, but it seems to be associated with more recalcitrant disease, often being discovered when initial surgical treatment fails to offer respite(3). Definitions of CRS vary depending on which international guideline is considered. The European

Position Paper on Rhinosinusitis and Nasal Polyposis 2012 updated from 2007 (EPOS)(4), the Canadian Clinical Practice Guidelines(5), the Rhinosinusitis Initiative (RI)(6), the Joint Task Force on Practice Parameters (JTFPP)(7), the Clinical Practice Guideline: Adult Sinusitis (CPG:AS)(8), and the British Society for Allergy and Clinical Immunology (BSACI)(9) are some of the current expert guidelines issued over available evidence and have issued varied definitions of CRS and its relationship to AR. Persistent AR could be included under the CRS definition: inflammation of the nasal and sinus mucosa of over 12 weeks duration. AR can be viewed as one pathway of sinonasal mucosal inflammation, that shares the same effector cells, cytokines and inflammatory mediators active in CRS(4). For the purposes of this thesis, AR is considered as a subset of CRS as in our experience over the last decade or so, we feel that the role of allergy in rhinosinusitis is underestimated. CRS can be further subdivided into with and without nasal polyposis. A subtype of CRS (usually with nasal polyposis) is fungal rhinosinusitis, which in its classical form has eosinophilic mucin with fungal hyphae and positive skin/blood tests for fungal antigens. The case for a fungal hypothesis in the aetiopathology of CRS, however, is tenuous at best. CRS with nasal polyposis (CRSwNP) is a distinct pathological subtype with a greater burden of symptoms and a higher relapse rate after treatment than those without polyposis(10). In spite of this observation, the actual distinction between the two disease phenotypes is less clear. The currently held view is that of distinct pathophysiological mechanisms leading to the predominance of one phenotype over the other with a more eosinophilic driven immunology underpinning those with nasal polyposis and possibly superantigens(11) and a greater role of neutrophils, bacterial infections and remodeling of the ostiomeatal complex in CRS without nasal polyposis(12).

Figure 1: Multifactorial nature of chronic rhinosinusitis



The above figure shows the multifactorial nature of CRS. The symbols illustrate the multiple factors implicated or investigated so far in CRS as opposed to direct causation and in certain cases such as atopy and allergic rhinitis, the relationship with chronic rhinosinusitis is far from clear. The detailed relationship between these factors and CRS and its phenotypes and endotypes is explored further in this chapter. Abbreviations – CRS, chronic rhinosinusitis; NP, nasal polyposis; OMC, ostiomeatal complex; *S aureus*, staphylococcus aureus.

We have for the purposes of this thesis chosen the definition of CRS as given in the EPOS guidance of 2007 and 2012(1, 13). CRS was defined as presence of two or more symptoms; one of which was nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip). In addition, the subject could have facial

pain/pressure, a reduction or loss of smell for at least 12 weeks and nasal endoscopy performed to evaluate the presence and severity of nasal polyposis as detailed in the methods section. A diagnosis of allergic rhinosinusitis was made according to the Allergic Rhinitis and its Impact on Asthma guidelines(2). We also evaluated those with aspirin intolerant rhinosinusitis (AIR) with and without asthma using both clinical assessment and nasal L-ASA (lysine aspirin) testing (see methods). Patients with AIR are known to represent the more severe and treatment resistant spectrum of CRSwNP, and with the limited avenues of therapy that are open to such patients, pharmacological optimization of current regimens is paramount to restoring quality of life.

EPIDEMIOLOGY AND BURDEN

CRS is one of the commonest chronic diseases affecting the developed world and is the commonest chronic respiratory disorder in the United States and Europe(14). In the 1997 National Health Interview Survey conducted by the U.S. Census Bureau for the National Centre for Health Statistics, Centres for Disease Control and Prevention, CRS ranked second among chronic health conditions(14). According to the National Institute of Allergy and Infectious Diseases Fact Sheet, CRS is the most commonly reported chronic disease, affecting nearly 32 million in the United States in 1997. In a study involving 73,364 subjects in Canada, the prevalence of CRS, defined by the question “Has the patient had sinusitis diagnosed by a health professional lasting for more than 6 months?” ranged from 3.4% in male to 5.7% in female subjects(15). On screening a non-ENT population, which may be considered representative of the general population in Belgium, 6% of subjects suffered from chronic nasal discharge(16). In the Skovde population-based study in Sweden , a

prevalence of 2.7% of nasal polyposis was estimated(17). A postal questionnaire survey sent to 4300 respondents in Southern Finland, estimated the prevalence of nasal polyps at 4%(18). Using a disease-specific questionnaire, the prevalence of nasal polyposis was reported as 2.1% in a sample of more than 10000 French people(19). A comparative study looking at secondary care patients in the otolaryngology outpatients in Aberdeen and Trinidad found a similar prevalence of CRS of approximately 9%(20). Almost a third of general practice referrals in adults and up to 50% of referrals in children are related to otolaryngological pathologies(21). More recently, in a postal questionnaire administered to 57,128 responders living in 12 European countries based on the EPOS 2012 criteria, the estimated disease prevalence was 10.9% (range 6.9-27.1)(22). The crux of all these data seems to be that a history driven approach seems to over-estimate prevalence and an endoscopy/physician driven approach underestimate it.

CRS is a disease affecting all age groups but, perhaps with an onset in young adulthood(23). While some studies suggested a slight female preponderance, a large community-based study by Klossek and coworkers demonstrated no sex predilection(19). Pilan and coworkers showed a mean age of onset in their cluster-sampling population study of 2000 adults in Sao Paulo of 40 (SD 21) years; 45.33% of whom were male(24).

As a disease, CRS with and without nasal polyposis is prone to trivialisation by patients themselves and under-diagnosis by first contact practitioners like primary care physicians. CRS is diagnosed and managed by a wide variety of practitioners, including primary care physicians, otolaryngologists, pulmonologists and allergologists. It is one of the most common reasons for presenting to primary care and accounts for 21% of all adult antibiotic prescriptions in the US(25, 26). Adult

CRS triggers approximately 18 to 22 million annual physician visits and 545000 annual emergency department attendances in the United States(27). In a survey using the Medical Outcomes Short Form 36-Item Questionnaire, patients with CRS had worse quality of life and more impaired social functioning than chronic conditions such as chronic obstructive pulmonary disease, angina, congestive heart failure, and back pain(28). More recently, in the first and one of the largest multicentre epidemiological studies in Europe of its kind, the population prevalence of CRS was estimated at 10.9% (range 6.9-27.1%)(22). In another recent study by Global Allergy and Asthma European Network there was a strong demonstrable association between CRS and asthma (adjusted OR: 3.47; 95% CI: 3.20-3.76) across all age-groups. The association with asthma was stronger in those reporting both CRS and allergic rhinitis (adjusted OR: 11.85; 95% CI: 10.57-13.17)(29). While the strong link between CRS and asthma has been established, its putative associations with chronic cough syndrome, depression and even lung malignancy are less well appreciated(30-33). CRS is diagnosed and managed by a wide variety of practitioners, including primary care physicians, otolaryngologists, pulmonologists and allergologists. CRS accounts for billions of dollars spent in healthcare costs, though this is likely to underestimate indirect costs due to lost productivity, sickness absenteeism and concomitant illnesses like asthma and allergic rhinitis(26, 30, 34).

In a multicentre European study looking at the natural evolution of aspirin induced rhinosinusitis with asthma, Szczeklik and coworkers demonstrated that the age of onset of nasal symptoms was in the fourth decade on average and that anosmia was a predominant feature in 55% of patients(35). This was followed by bronchial symptoms approximately 2 years later and aspirin intolerance developed further on. Hastan and coworkers demonstrated in a large cohort of over 50000 subjects that

CRS was linked with an increased prevalence of late onset asthma(22). This was confirmed by Jarvis et al who in a Global Allergy and Asthma Network of Excellence study surveyed 52000 subjects and found an overall age-adjusted incidence of asthma in CRS of (odds ratio 3.5, 95% CI: 3.2-3.8)(29).

Longitudinal 8.8 year follow-up data from the European Community Respiratory Health Survey demonstrated that irrespective of atopic status, merely having rhinitis led to an increased relative risk of developing lower airways disease of 2.71 (95% CI:1.64–4.46)(36). Moreover persistent symptomatology as deemed by the ARIA® criteria were also associated independently with the development of lower airways disease leading to the conclusion that CRS is an umbrella term for all sinus disease but actually which is a constellation of closely associated pathologies manifesting varyingly, which are yet to be fully understood.

PATHOGENESIS

CRS is a multifactorial disease with complex interplay of host and environmental factors that are not fully elucidated yet (Figure 1). In a minority of cases CRS is a manifestation of a systemic illness such as cystic fibrosis, primary ciliary dyskinesia, and hypogammaglobulinaemia, but in the vast majority of cases, CRS is deemed idiopathic.

Genetics

As mentioned above there are a few disorders like cystic fibrosis and Kartagener's syndrome that are strongly associated with a tendency towards CRS and in particular CRSwNP(37). However, supportive data for a genetic 'sensitisation/predilection' are lacking. Lockey and coworkers studied 4 members of a Mennonite family to

elucidate an autosomal recessive basis for the transmission of aspirin intolerant rhinosinustis with asthma(38). Some HLA haplotypes have been implicated in the a priori risk of developing nasal polyposis(39). It is also possible that many genes involved in the pathogenesis, course and severity of allergic rhinitis and asthma are common to CRS(26). Using genome-wide expression microarrays Stankovic and coworkers demonstrated five genes likely to be involved in the pathogenesis of CRS and aspirin sensitivity(40). A familial preponderance has also been demonstrated in certain studies. Using a case-control study design Alexiou and coworkers showed in a 102 patients, a 13% familial prevalence of nasal polyps as compared to population controls(41). However, in the vast majority of idiopathic cases a clear genetic component is not defined as evidence by a lack of increased prevalence in monozygotic twin studies(9).

Molecular mechanisms and Endotypes

There is an increasing interest in identifying disease phenotypes in CRS, e.g. CRSwNP, CRSsNP and aspirin intolerance. For e.g. presence of ostiomeatal complex obstruction is considered by many to be a surrogate of disease severity and overall burden and may predict a worse outcome(42). CRS with nasal polyposis (CRSwNP) is a distinct phenotype with a greater burden of symptoms and a higher relapse rate after treatment than those without polyposis(10). But merely identifying these clinical phenotypes has proven to be of limited value from a long-term perspective, as this does not provide a holistic understanding of the cellular mechanisms underpinning CRS. Moreover, as yet there are no specific treatments tailored for each phenotype. As mentioned before, CRS is an umbrella term for a collection of various diseases. There is a growing trend towards ‘endotyping’ as well as phenotyping i.e. defining subtypes based on a distinct pathogenetic mechanism or

biomarkers(37). Such endotyping may help define future treatment responses, such as differential corticosteroid response between CRSwNP and CRSsNP, patients with a marked *Aspergillus* IgG burden, aspirin intolerance and desensitisation. We know currently that there are key molecular and cellular differences between the major phenotypes of CRSwNP and CRSsNP. In a landmark paper by Van Zele and coworkers, characteristic differences were found between CRSwNP, CRSsNP and cystic fibrosis polyposis patients in terms of effector cell predominance, Th1/Th2 polarization and levels of TGF- β (43).

Table 1. Characteristics of CRS endotypes

Endotype	CRSwNP	CRSsNP
Anatomical factors	Nasal polyposis, blocked ostiomeatal complex	No associated anatomical factors
QoL and disease burden	Worse QoL and symptoms, relapses common, more therapy-recalcitrant	Better QoL and disease burden
Type of cell mediating disease	Th2 predominant	Th1 predominant
Cytokines	Raised IL-5, eotaxin, IgE Low TGF- β	Raised IFN-gamma, TREG and TGF- β
Abbreviations: CRSwNP, chronic rhinosinusitis with nasal polyposis; CRSsNP, chronic rhinosinusitis without nasal polyposis; QoL, quality of life		

However, the above diagram may be not be necessarily accurate in all patient populations, as recent evidence has shown that there is a considerable overlap in these mechanisms in Asian patients(44). More recently, there has been interest in the levels of fibrosis in CRSwNP and CRSsNP, whereby in nasal polyps there is lesser fibrotic change, upregulation of metalloproteinases such as MMP-7 and MMP-9, more tissue oedema and a defect in T cell regulation (Treg)(26, 45). Despite these ethnic differences, it is clear that eosinophilic activation and IL-5 induced reduction of apoptosis plays a crucial role in the activation of specific adhesion receptors and possibly in conjunction with *Staphylococcus aureus* enterotoxins, which may serve as superantigens, induce a cascade of T and B cell activation and amplification of the inflammatory cascade(46-48).

***Staphylococcus aureus* enterotoxins or ‘Superantigens’**

It is generally accepted that IL-5 and eotaxin secretion in CRS orchestrates the chemotaxis, activation, migration and further release of eosinophilic mediators in inflamed tissues and a reduction in cellular apoptosis(49, 50). *Staphylococcus aureus* is one of the commonest bacteria found in patients with CRS and it much more commonly found in CRSwNP(37, 51). *S aureus* elaborates a number of powerful factors that are capable of initiating a Th2 response through staphylococcal enterotoxins (SE) or other proteins and are capable of strikingly amplify the inflammatory cascade in CRS(49, 52). In vitro studies have demonstrated this modulatory effect on Th1 and Th2 cytokines (IFN-gamma, IL-2, IL-4, IL-5, IL-10 and IL-13) when polyp tissue was exposed to SEB(47). Van Zele and coworkers

showed that nasal polyp homogenates in which *S. aureus* enterotoxin specific – IgE antibodies were detectable had significantly greater concentrations of IgG, IgG4 and IgE than did those without(49). Moreover, it is known that SEA and TSST-1 (examples of staphylococcal enterotoxins) can induce a rapid polyclonal response to multiple allergens, modulate the allergic response by augmenting isotype switching and IgE synthesis(53). Further, there may even be a link between eicosanoid metabolism and SE with the demonstration of upregulation of cysteinyl leukotrienes, leukotriene B4 and lipoxin A4 in polyp tissue from those with an immune response to *S. aureus* enterotoxins as compared to those who were negative for *S. aureus* enterotoxin – IgE(54). This is potentially of interest as increased synthesis of pro-inflammatory leukotrienes and decreased synthesis of anti-inflammatory prostaglandins (PGE2) have been proposed as a mechanism not just for aspirin-sensitive nasal polyps but also aspirin-tolerant CRSwNP(55). However, while multiple direct and indirect data are available to substantiate this rather interesting hypothesis, a direct causal link is yet to be clearly established, and the putative role of SE in informing therapeutics is to be explored.

Microorganisms and the Immune barrier hypothesis

Acute rhinosinusitis (ARS) is triggered by infectious organisms, with perpetuated inflammation as the underlying basis of the disease, modulated by local anatomical and other host factors, which play a role in disease perpetuation, progression and recurrence(1). A normal milieu in the host in the form of normal anatomy, histology and tissue function essentially precludes recurrent infections, and there is a vicious cycle of repeated infections leading to changes in tissue function and cascades of inflammation that are responsible for the remitting relapsing nature of the

disease(56). CRS is not to be considered to arise from ARS and has a distinctive pathophysiology underpinning it. Indeed, the role of microorganisms in CRS is unclear at present with a rekindled interest as a result of *S aureus* enterotoxins as described above.

With the advent of culture-independent techniques, a wider variety of organisms can now be detected, although it remains to be seen if there is a clear role within the pathological framework of disease(57). In particular, it has been recently reported that both bacterial and fungal biofilms are present more commonly in CRS than in normal controls(58, 59). These biofilms have also been demonstrated in CRSsNP and have been shown to respond to macrolide therapy rather than traditional corticosteroid treatment(60). Basically, biofilms may provide a shielded environ for a reservoir of microbiota in which they are protected from host defenses and treatment, and may play a role in refractory CRS.

Similarly, the role of fungi in CRS is a matter of continued debate. Fungi are ubiquitous in nature and have been detected in the nasal mucosa of both patients with CRS and normal subjects(61). Shin and coworkers found that patients with CRS showed exaggerated humoral and cellular responses, both Th1 and Th2, to common airborne fungi, particularly *Alternaria* using peripheral blood monocytes(62). Lastly, patients with CRS demonstrated excessive eosinophilic inflammatory change in the nasal mucosa in the absence of mould-specific IgE reactivity(61). This led to the suggestion of a 'Fungal Hypothesis' in CRS. Disappointingly, in a large multicentre randomized clinical trial by Ebbens and coworkers, they failed to demonstrate efficacy of topical Amphotericin B in patients with CRS using total visual analogue scale (VAS) score and nasal endoscopy score as their primary outcome measure(63). Similarly, in a prospective randomized controlled trial, Gerlinger and colleagues

failed to demonstrate an improvement in postoperative CT scores, clinical symptoms, or quality of life measures in CRS using amphotericin B nasal spray(64). There is perhaps a broader repertoire of in vitro data to support the role of fungi in the aetiopathogenesis of allergic rhinosinustis(65). This along with some restricted clinical responses seen still maintain the likelihood that a fungal endotype is possible, although a wider role in CRS is currently questionable. Areas of future research interest in this arena include immunomodulation of host response and a chitin-induced Th2 response secondary to direct colonization or biofilm formation(1). Chitin ($(C_8H_{13}O_5N)_n$) is a long-chain [polymer](#) of N-acetylglucosamine and is an abundant polysaccharide type found in the cell walls of fungi, insects, and parasitic nematodes. Innate immune host defense against chitin-containing pathogens include production of chitinases. There is some evidence that an increased expression of acidic mammalian chitinase in chronic rhinosinusitis with nasal polyps may influence the Th2 mediated inflammation in the upper respiratory tract(66).

Lastly, the effect of these microorganisms or allergens is enhanced due to barrier breakdown in the form of epithelial tight junctions, which are critical in controlling paracellular flux of substances(37, 67). The human nose is constantly exposed to a wide variety of environmental insults and acts as a gatekeeper to exogenous irritants and noxious stimuli(68). Epithelial tight junctions are delicately regulated by complex interactions between a variety of adhesion molecules and represent a dynamic ever-changing interface between the human body and its surroundings(67). Their disruption represents a key link in the etiologic chain of causation in CRS. Similarly, several studies have implicated a reduction in the responsiveness of Toll-like receptors which are sinonasal epithelial receptors that play a critical role in innate and acquired immune responses(69). In particular, upper airway cells are host

to TLR2, TLR3, TLR4 and TLR9, which may play a role in mediating host inflammation, with consequential derangements contributing to the final common pathway of CRS(70). In particular, TLR 2 and TLR9 have been implicated in recalcitrant rhinosinusitis and in patients with early polyp recurrence post FESS(71).

Allergy

Current international consensus is that the role of allergy in CRS is limited and unclear(9, 72). A number of studies have reported an increase in the prevalence of atopy in CRS(1, 73, 74). Friedman reported a 94% prevalence of atopy in his cohort of patients undergoing sinus surgery(73). Authors have reported a prevalence of 30-94% with regard to patients with CRS with positive skin prick tests(72, 75-78).

There are data contrary to this with authors reporting no increase in the prevalence or severity of CRSwNP in patients with allergies(79, 80). A more population-based prevalence is reported as much lower in the range of 0.5 to 4%(81-83). In our institution, we have a high background prevalence (approximately 50% - see also Chapters 3 and 4) of allergic symptoms and atopy in our patient cohort of CRS(84-86). Interestingly, this is quite similar to a large European survey by GA2LEN in 52000 people, reported a 56.7% prevalence of allergic symptoms in those who fulfilled the EPOS 2012 criteria for CRS(22).

What is clear is that both diseases share a common denominator of inflammation, similar symptomatology, are both demonstrating a rising global trend, and frequently co-exist in the same patient. Baroody and coworkers demonstrated the concept of a unified inflammatory milieu for nasal and sinus mucosa in two experiments, one of which was a double blind, crossover, randomized, placebo-controlled study in 20 allergic subjects out of season, to show that allergen challenge to the nasal mucosa resulted in an allergic response from the maxillary sinus mucosa(87, 88). Lin et al

found a higher rate of surgery, increased disease severity and higher medication use in CRS patients with concomitant allergic rhinitis(78). These data are similar to other authors who have shown an increase in symptom severity in patients with concomitant CRS and allergies using validated symptom scores such as SF-36, Rhinosinusitis Disability Index and SNOT-20(89-91). Lastly, a systematic review by Contreras and coworkers demonstrated a mixed picture with a probable high concomitant prevalence of atopy with CRS, but high quality evidence was still lacking(72).

Aspirin sensitivity

Of note, 5% to 8% of patients with CRSwNP will be intolerant to aspirin (acetylsalicylic acid) and non-steroidal anti-inflammatory drugs (NSAIDs) and almost invariably have associated asthma. This trio of disorders is often referred to as the 'aspirin triad' or 'Samter's triad'(92). It is known by several acronyms and names such as aspirin-induced/intolerant asthma (AIA), aspirin-induced/intolerant rhinosinusitis (AIR), aspirin sensitive asthma (ASA), aspirin hypersensitivity and aspirin exacerbated respiratory disease (AERD).

A meta-analysis showed that the prevalence of aspirin sensitivity in the general population may be as high as 22% if oral aspirin challenge tests are used as the diagnostic gold-standard instead of patient history(93). Moreover, there is almost a 100% cross-sensitivity to common over-the-counter NSAIDs(93, 94). Aspirin sensitivity affects about 10% of adults with chronic asthma and in patients with aspirin sensitivity, 36% to 96% have nasal polyposis as evidenced by endoscopic or radiographic changes(1).

The pathogenetic mechanisms of AIR are complex and not fully understood yet. Common hypotheses include an imbalance in the arachidonic acid pathway causing

an overproduction in leukotrienes in genetically predisposed phenotypes(95). It is postulated that the inhibition of the COX1 enzyme by aspirin (or related substances) leads to subsequent inflammatory cell activation, and the release of both lipid and non-lipid mediators(96). A significantly lower generation of PGE2 and COX2 expression in the nasal and sinus mucosa has been reported(97, 98). This could contribute to the development of the severe eosinophilic inflammation characteristic of AIR patients. AIR usually presents in the third and fourth decades and is more common in females and in non-atopic individuals(9). It may present initially as non-specific rhinorrhoea and nasal congestion and progresses over the next decade or so to aspirin sensitivity, asthma and nasal polyposis(99).

Typically, the presence of aspirin intolerance makes the upper and lower airways disease severe, persistent and treatment resistant(35). Patients with AIR have a higher frequency of hospitalisations and emergency department visits than tolerant patients(100). Their illness is recalcitrant to medical and surgical treatment and they are often either steroid dependent or unresponsive(9). In a pan-European survey of AIR, inhaled corticosteroids or oral corticosteroids were used by 80% of afflicted patients with a mean daily dose of 8 mg of oral prednisolone(35). Endoscopic sinus surgery outcomes are also less successful in AIR patients compared with aspirin tolerant patients, with more frequent and earlier relapses(101).

The diagnosis of aspirin sensitivity relies upon either a clear history of two or more aspirin/NSAID induced reactions and/or on aspirin challenge, which can be oral, inhaled or intranasal(9). Establishing a diagnosis of aspirin intolerance is important. It provides the patient with a host of common drugs that must be avoided, diagnoses a particularly severe and recalcitrant form of disease phenotype and allows a choice of specific therapy such as leukotriene modifiers or aspirin desensitisation(1).

Oral aspirin challenge with placebo control is widely used, but is time consuming (it can be three days long), and may provoke severe bronchospasm and anaphylaxis.

Moreover, a negative oral challenge cannot by itself rule out AIR. Indeed the possibility of false-negative responses increases when diagnostic provocation tests with aspirin are carried out in asthmatics being treated with corticosteroids or during symptom-free intervals(94). However, when endpoints such as a 20% reduction in forced expiratory volume in 1 second (FEV1), or characteristic extrathoracic symptoms (such as severe rhinorrhoea and nasal congestion), are used oral aspirin challenges have a sensitivity of 89% and specificity of 93%(102).

Inhalation or nasal challenge with lysine aspirin (L-ASA) is becoming the preferred method. L-ASA is more soluble than aspirin (40% versus 0.3%), is non-irritant, and is well tolerated when inhaled(94). Nasal challenge with L-ASA is similarly sensitive and specific (95.7% and 86.7%, respectively), but a negative test does not exclude aspirin sensitivity(103). Its negative predictive value is as low as 78.6% and an oral challenge is recommended after a negative nasal test(103). Lastly, irrespective of challenge results, aspirin sensitivity should always be suspected in patients with severe nasal polyposis, especially those with recurrent polyps and steroid dependent refractory asthma(9).

Nasal Anatomy – gross, microvascular and molecular anatomy

There are some purported nasal anatomical factors which have been deemed abnormal or are high risk for the development of CRS such as concha bulllosa, paradoxical middle turbinate, deviated nasal septum, a displaced uncinat process, accessory sinus ostia, and atypical ethmoidal air cells (104). Several authors have found no correlation between sinus opacification, nasal symptom scores and normal

sinonasal anatomic variants(105-107). Correction of these abnormalities may be carried out as part of a more functional sinus surgery for better access.

The microvascular anatomy of the nose provides a more clear understanding of the potential regulatory mechanisms for nasal congestion and decongestion in physiology and pathology(108).

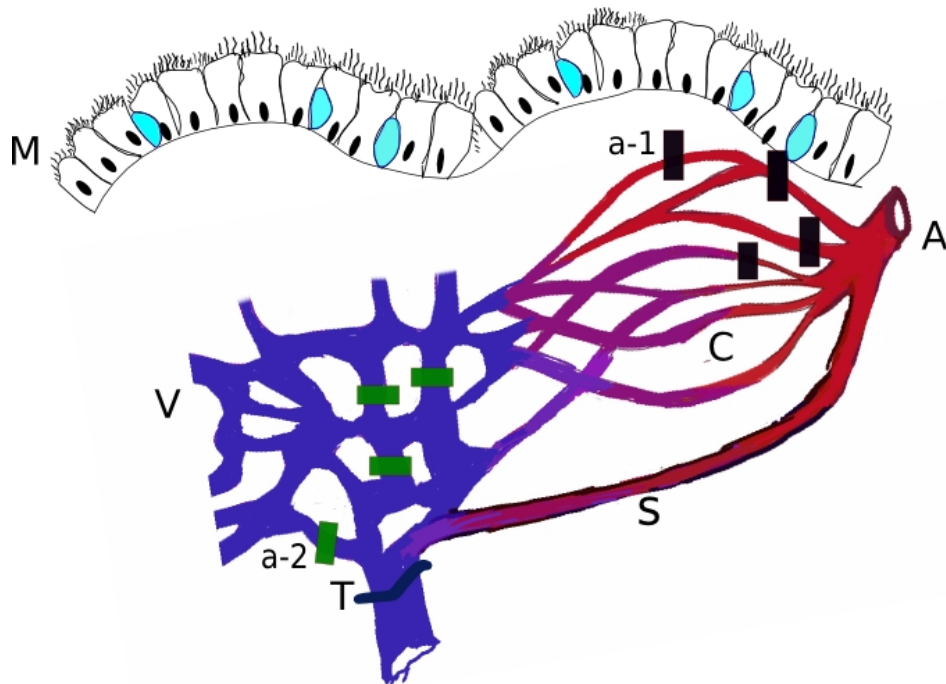


Figure 2: Schematic to demonstrate human nasal microvascular anatomy and differential adrenoceptor response.

M = nasal respiratory-type ciliated pseudostratified epithelium with goblet cells (blue), a-1 = alpha-1 adrenoceptors, a-2 = alpha-2 adrenoceptors, A = arterioles, C = capillaries, S = arterio-venous shunts, V = venous sinusoids, T = throttle veins. The nasal mucosa (M) is made of respiratory-type pseudostratified columnar epithelium and goblet cells (blue). The arterioles (A) feed an abundant plexus of submucosal capillaries (C), the density of which is required to supply nutrients, transport oxygen and remove toxins for one of the most metabolically active epithelium in the human body. There are however, some 'anomalies' when compared to the rest of the upper

airways that makes the nose unique(109). The capillaries have extensive fenestrations on the epithelial side, allowing water to escape for evaporation acting as an air conditioner. There is an extensive unique network of arteriovenous shunts (Figure 2 - S) that bypasses the capillary network. This is similar to other highly vascular beds like the fingernail bed. Uniquely, the human nose (like other mammalian noses) has capacitance vessels or venous sinusoids (V) enclosed in an osseous cavernous cover that serve to engorge and expand in response to stimuli and cause congestion and decongestion, the so-called nasal cycle(110). It is also likely that the shunts and capacitance vessels increase the capacity of the nasal mucosa to act as an air conditioner.

Adrenoceptor Regulation

Adrenergic receptors mediate the effects of adrenaline and noradrenaline and are virtually involved in the regulation of every organ system in the human body. In the early 1900s adrenoceptors were identified through tissue response methods. The exemplary division of these into alpha and beta-adrenoceptors in the 1960s was of critical importance and since then their importance in modern day medicine and research is self-evident(111). It was the advent of radioligand binding assays that represented the next major advance in the classification and study of adrenoceptors(112). Data from these assays and second messenger studies have shown that there are at least six subtypes of alpha adrenoceptors alone(113). Stafford-Smith and coworkers demonstrated using in situ hybridization of nasal turbinate samples the prevalence of alpha_{2c}-adrenoceptors on the venous sinusoids and arteriovenous shunts and postulated that a selective alpha_{2c} agonist may be of value in providing decongestion whilst avoiding side-effects due to more generic receptor effects(114, 115). Corboz and colleagues demonstrated that alpha-2 adrenoceptors

predominate on the venular side of the nose while alpha-1 adrenoceptors on the pre-capillary side(116). This was in keeping with a prior study by Johannssen and coworkers that constriction of human nasal mucosal arteries was alpha-1 mediated(117). This led to the suggestion that a selective alpha-2 agonist could potentially influence congestion without affecting nasal mucosal blood flow, which is considered to be one of the key links in the chain causing rhinitis medicamentosa(118). These studies used oxymetazoline which has an approximately 5:1 ratio effect on alpha-2 vs. alpha-1 adrenoceptors(113). However, this is in conflict with what Ichimura and coworkers have demonstrated previously that the predominant adrenoceptor in the nose is an alpha-1 adrenoceptor(119). There may be an issue with size of tissues used, incubation techniques and experimental methodology, which may account for these effects. The reality is that there are no real life studies that have evaluated these effects in vivo and there is a wide chasm currently before any of this can be translated into daily practice.

The human nose is under tonic sympathetic control by post-ganglionic fibres emanating from the stellate ganglion in the neck and reaching the nose along arteries. Although there is an abundance of adrenergic innervation to these blood vessels (predominantly alpha-1), there is also sparse cholinergic innervation to the glands(120). While it is likely that a series of complex interactions are responsible for the fine regulation of nasal blood flow in health and disease, in simple terms, increasing the rate and duration of sympathetic nerve impulses to the nose causes vasoconstriction with the reverse effect i.e. decongestion in those who undergo cervical sympathectomy. Based on current evidence, the predominant adrenoceptor type in the superficial pre-capillary sphincters seems to be the alpha-1 subtype and on the deep venous sinusoids, the alpha-2 subtype(116, 121). Thus a selective alpha-

2 adrenoceptor would potentially have a higher therapeutic index, with a high efficacy to safety ratio, by causing contraction of the venous sinusoids but sparing an ischaemic effect on the nasal mucosa which has been hypothesized as a potential cause of rebound congestion. While one may theorize the existence of significant cross-talk between inflammatory mediators and adrenoceptors, the actual evidence in CRS is sparse(122). Kunkel and coworkers proposed a reduction in the numbers of beta-adrenergic receptors in the nasal mucosa of patients with nasal polyps and allergic rhinitis compared to healthy controls, raising the possibility of the human nose being a mirror to the bronchial mucosa(123). Indeed, in a study by Van Megen and coworkers in allergic rhinitis (not CRS), no significant change in the nasal alpha-adrenoceptor affinities or densities was demonstrated when compared to healthy controls(124). Moreover, animal studies may not be truly translatable into human subjects readily, given the difference in adrenoceptor profiles, for e.g. pig nasal mucosa seems predominantly under alpha-2 adrenoceptor control as opposed to the human nasal mucosa(125).

The link between the upper and lower airways

Some lessons can be extrapolated from research performed in the lower airways. This is due to the fact that rhinitis and asthma are clinically overlapping manifestations of a single unified airway disease which share many pathological and epidemiological characteristics, particularly in allergic rhinitis(2, 126). Given that the nose, sinuses and lower airways are all lined by similar ciliated pseudostratified columnar epithelium, resting upon a rich submucosa of vessels, mucous glands, fibroblasts, inflammatory cells and nerves, this is hardly surprising. Moreover, similar inflammatory mechanisms

underpin both disease processes, with a Th2 driven inflammatory cellular cascade releasing cytokine mediators such as IL-4, IL-5 and eotaxin found in both nasal and bronchial mucosa(127, 128). Indeed, treating upper airway inflammation has been shown to have a downstream effect and improve lower airway outcomes including airway hyper-responsiveness and pulmonary function(129). This link between upper airway inflammation and bronchial hyperreactivity is not limited to merely allergic rhinitis, however, and there is a risk of developing asthma with any rhinosinusitis irrespective of the presence of atopy. In a recent large community-based study in Europe by Global Allergy and Asthma European Network there was a strong demonstrable association between CRS and asthma (adjusted OR: 3.47; 95% CI: 3.20-3.76) across all age-groups. The association with asthma was stronger in those reporting both CRS and allergic rhinitis (adjusted OR: 11.85; 95% CI: 10.57-13.17)(29).

Asthma was historically thought to be associated with an intrinsic defect of β_2 -adrenoceptor function in airway smooth muscle that tipped the balance towards bronchoconstriction. Since then, there has been a shift in thinking to a more inflammation based approach in asthma, and the upper airways have similarly followed suit(2, 130). Despite this shift, there remains a reasonable body of evidence for a neural basis of disease with regard to beta-adrenergic receptor polymorphisms in asthma, although as mentioned before a similar understanding is currently lacking in rhinology. Of particular interest is that link between the neural control and inflammatory processes which are yet to be fully elucidated. In asthma, it has been recognized that genetic factors, evidenced by greater incidence rates in family members of asthmatics, include polymorphisms of a number of genes involved in atopy, airway inflammation and bronchial smooth muscle tone including the beta2-

adrenoceptor(131). There is some evidence in the literature that this may influence treatment dosages of corticosteroids(132). Palmer and coworkers demonstrated that the arginine-16 genotype of the beta-2-adrenoceptor predisposes to exacerbations in asthmatic children and young adults, particularly in those exposed to regular salmeterol(133). This may be explained by salmeterol-induced downregulation, impaired receptor coupling, and associated dampening of the response.

More recently, a landmark study by Hanania and coworkers showed that chronic administration of an antagonist at the beta-adrenoceptor in asthma, actually causes something unexpected, and they start acting as agonists instead and upregulate these receptors (134, 135). More importantly, this caused a downstream decrease in bronchial hyperreactivity using methacholine challenge which is a surrogate for lower airways inflammation. This was a clear demonstration of how tweaking neural networks could potentially influence airway inflammation. If this held true in the upper airways, what this could mean was that the chronic administration of an alpha-adrenoceptor antagonist in AR or CRS could help reduce nasal hyperreactivity.

Short and coworkers, however, in a double blind randomized clinical trial could not demonstrate an effect of chronic dosing of propranolol on methacholine challenge in asthmatics(136). Despite the above conflicting results, this remains an area of potential growth and further studies in the nasal mucosa using an alpha-receptor blocker such as prazosin might have an influence on nasal mucosal reactivity, which is a ubiquitous feature of rhinosinusitis.

Moreover, alpha-agonists are one of the most efficacious drugs in relieving congestion which is a principal symptom of CRS and one that impairs QoL more than any other(1). However, their use is currently restricted due to the development of tachyphylaxis at the receptor manifesting as decreased efficacy and more

worryingly the development of rebound congestion i.e. rhinitis medicamentosa(137). In the lung, it has been shown by Tan and coworkers that administration of a single dose of oral corticosteroid will rapidly reverse beta-adrenoceptor subsensitivity by upregulating their concentrations dramatically in the airway smooth muscle cells(138). We also know that alpha adrenoceptor downregulation is a rapid phenomenon and more than 50% of receptors will downregulate with a half-life of 2.5 hours(139). We therefore explore the assumption that corticosteroid administration in the nasal mucosa might do the same and prevent or delay the onset of tachyphylaxis and rebound congestion. This is one of the major themes explored in this thesis.

TREATMENT OPTIONS

The mainstay of treatment for CRSwNP and CRSsNP is medical with surgery playing a role in providing improved access to medical therapy, dealing with severe or recalcitrant disease, steroid-resistant disease, AIR, and last but not least dealing with complications of CRS such as mucocoele formation, intraorbital and cranial complications(1). Of course, surgery represents an extremely effective treatment option and has been shown to improve outcomes, quality of life scores and prevent morbidity(37, 140). Dalziel and coworkers performed a systematic review and metaanalysis of more than 10000 patients spanning two decades concluded that the vast majority of patients derived benefit from functional endoscopic sinus surgery (FESS) and open procedures with a very low complication rate (1.4% for FESS)(141). In a large UK based prospective cohort study titled the National Comparative Audit in CRS, significant improvements in SNOT-22 scores were demonstrated up to 3 years post-surgery with greater benefit in those with CRSwNP

than CRSsNP(142). However, revision surgery was indicated in 3.6% of patients at 12 months and 11.8% at 36 months(142, 143). A randomized trial by Ragab and coworkers comparing long-term medical management with surgical treatment in patients with both CRSwNP and CRSsNP using a vast array of objective (polyp scores, NO, PNIF and mucociliary clearance) and subjective measures such as VAS, SNOT-20, SF-showed that both treatments significantly improved almost all parameters of CRS ($P < .01$), with no significant difference between groups(144). In summary, surgery and medical therapy must be considered complementary in CRS.

Corticosteroids

The mainstay of treatment for CRS remains long-term intranasal corticosteroids (INCS)(1, 9). There is an extensive evidence in this regard, which has been previously summarized, systematically reviewed and meta-analysed(1, 145, 146). INCS have demonstrated unparalleled efficacy based on subjective and objective outcome measures such as polyp size, endoscopy score, symptoms score, nasal airway patency measures such as PNIF; improved QoL; reduced surgical events and improved post-surgical outcomes(1, 9, 145). They are also known to significantly improve ocular symptoms in those who have allergic CRS(147). No evidence exists currently for the recommended use of one INCS over another in terms of efficacy. A twice-daily dosing regime seems more effective than once daily administration(148). There is also a significant effect of the technique of administration on the efficacy of nasal based therapy and patient education plays a key role in this illness(9, 149). The predicament with chronic maintenance treatment with INCS is the slow attrition of efficacy as evidenced by the chronic remitting relapsing nature of CRS(150). This

may be hypothesized to occur due to a lack of effect of INCS on the ostiomeatal complex and sinus drainage requiring multiple medical polypectomies(9). Clearly, this is the reported experience of many practitioners treating CRS, however, evidence in favour of using an initial oral corticosteroid induction was lacking until recently, and these data have been presented later in this thesis(85).

In particular, there is a remarkable lack of safety data in the literature on INCS in CRS patients. INCS are highly lipophilic compounds and this diminishes the solubility of these compounds in the water-dominant nasal mucosa, reducing direct systemic absorption from the nasal cavity. Indeed, the reported systemic bioavailability of INCS in the literature varies between 1-50% and clearly with ease of availability over-the-counter, there is a risk of systemic adverse effects(151, 152). This assumes greater importance as many patients with CRS have associated asthma and are on inhaled corticosteroids and in patients with AIR or steroid-refractory disease; these factors clearly are additive in terms of the overall steroid burden(9, 81). It is also well-recognised that in real life, it is uncommon for patients to have their inhaled and intranasal corticosteroids tapered or fine-tuned frequently. Indeed, in the experience at our institution step-down of therapy is rarely achieved, particularly in the community or even secondary care(153). So far most studies in CRS have focused on reported symptoms such as epistaxis, although the EPOS guidelines conclude that the existing literature in INCS has failed to show any significant systemic adverse effects(1). This assumption in the current guidelines must be taken with a note of caution. Most data in the literature is in the form of systemic detection after single pass metabolism. For example a study by Daley-Yates assessing systemic bioavailability of four consecutive 800 µg doses intranasal fluticasone aqueous spray given over 24 h in healthy volunteers concluded that even

at 12 times the normal dose was safe and that drops were 8 times less detectable than spray(154). This is likely to be an incorrect assumption, as drugs such as fluticasone are highly lipophilic and are mainly stored in the adipose tissue of the body due to a large volume of distribution and may have prolonged systemic effects despite a lack of detection in the intravascular compartment(153). Vargas and coworkers claimed that a lack of effect after 4 weeks of high dose fluticasone on a 250 µg ACTH stimulation test may be explained by the known insensitivity of this test, as 250 µg represents a supraphysiological dose of ACTH, with much lower doses of ACTH (i.e. 0.5–1.0 µg) being as effective in producing a stimulated cortisol response(155). Knutson and coworkers showed in healthy volunteers receiving intranasal fluticasone spray 200 µg daily for 1 week followed by 400 µg daily for a second week, that there was a 37% fall in 0800 h serum cortisol, a 24% fall in 24-h urinary cortisol, a 45% fall in serum osteocalcin as well as a 28% fall in peripheral blood lymphocytes glucocorticoid receptor mRNA expression, all of which were highly significant effects ($P < 0.001$)(156). Wilson and coworkers have shown in a study on healthy volunteers, that administration of intranasal fluticasone spray 200 µg daily compared with placebo resulted in significant HPA-axis suppression in terms of a 43% reduction of overnight urinary cortisol excretion, but not triamcinolone spray 220 µg(157). In summary, not only in children, but also in adults, practitioners must be aware that efficacy and safety monitoring must go hand in hand and that in CRS there is a potential for significant additive steroid burden.

There is evidence of short-term efficacy for oral corticosteroids in CRSwNP with a systematic review of 3 clinical trials by Martinez-Devesa and coworkers, but no long-term or frequent use data existed until this thesis data(158).

Antibiotics

There is little evidence to support the routine use of short-term antibiotics in CRS(1, 37). Two short-term studies exist in terms of using staphylococcal specific antibiotic treatment based on the SE superantigen hypothesis. The study by Schalek and coworkers evaluating patients undergoing FESS randomized to receive a post-operative 3-week course of oral anti-staphylococcal antibiotics in CRS did not show any additive benefit at 3 or 6 months in terms of endoscopy score or SNOT-20 QoL outcome(159). Van Zele and coworkers in another RCT looking at a 3 week course of oral Doxycycline 100mg daily for 3 weeks showed significant improvements in polyp size, nasal secretions and nasal inflammatory marker reduction but not QoL(160).

Most of the long-term data is in macrolide antibiotics due to their putative immunomodulatory effects(161). Macrolide antibiotics have traditionally been used for their antibiotic effects in acute rhinosinusitis and in exacerbations of chronic rhinosinusitis(162). In this regard they are as efficacious as other penicillin and non-penicillin antibiotics(1). However, recent years have seen an increasing interest in their anti-inflammatory actions(163). The first such report, was in patients suffering from diffuse panbronchiolitis experiencing marked improvement in their symptoms with low dose macrolide therapy, by Kudoh et. al.(164). Since then, many *in vivo* and *in vitro* studies have shown similar effects in cystic fibrosis, asthma, chronic rhinosinusitis, rheumatoid arthritis, cancer and coronary artery disease(165). It is to be noted that this is not a down-regulation of the immune system, and there is no evidence of immunosuppression(166). In fact, it is broad-based modulation of the biological inflammatory response without jeopardizing the host ability to combat infection. It is also independent of their anti-microbial activity; macrolides have shown excellent clinical efficacy even in the presence of resistant microorganisms at

lower than anti-microbial doses. In cystic fibrosis for instance, studies have shown a positive effect including a marked reduction of cytokine levels and significant improvement in lung function(167). Macrolides decrease pro-inflammatory cytokine secretion (IL-5, IL-8, GM-CSF etc.), inhibit neutrophil chemotaxis, adhesion, oxidative burst and increase apoptosis(166). They accumulate intracellularly and mediate a variety of effects presumably through the key proinflammatory transcription activator NF- κ B unlike glucocorticoids(168).

The first clinical study in the English literature to show benefit in CRS was by Hashiba and Baba(169), they showed a 71% improvement in symptoms after 12 weeks of clarithromycin (CLARI) 400 mg daily. In another longitudinal study using erythromycin 250 mg bid or CLARI 250 mg od, 70% of patients experienced significant improvement in their symptom scores, saccharine clearance time and nasal endoscopy grading(170). It is estimated clinically that the anti-inflammatory effect of macrolides is present at low doses (half of the anti-infective dose), and that it takes longer to manifest than the anti-microbial effect (50% by 4-6 weeks and full effect by 12 weeks)(171). Also, there seems to be a proportion of patients do not respond to therapy and this is usually apparent by 3 months. While this lack of response is poorly understood, indicators of good treatment response include normal IgE levels, High tissue IL-8 levels, small polyp size(163, 166).

Researchers have also found that macrolides reduce the levels of some tissue cytokines such as IL-8, a critical cytokine in the pathogenesis of CRSwNP(172, 173). IL-8, a potent neutrophil chemoattractant, is elevated in patients with CRS as opposed to allergic rhinitis (AR) and may be a key factor in the formation of NP(1). Indeed, in a prospective study patients with NP treated with low dose macrolide therapy, treatment responders experienced a significant fall in IL-8 levels as

compared to non-responders(173). They also had significantly higher baseline IL-8 values (231.2 vs. 88.1 pg.mL⁻¹, $P<.005$). Macrolides inhibit the production of superoxide and other free radicals by neutrophils in a dose dependent manner(174). Macrolides also reduce the activity of inducible nitric oxide synthase (iNOS), an enzyme that produces NO, a potent mediator of inflammation in the upper airways(165). In a comparative *in vitro* study, CLARI and prednisolone both reduced the levels of IL-5, IL-8 and GM-CSF in nasal epithelial cells(161).

Polyps can be classified pathologically into neutrophil predominant or eosinophil predominant. The majority of evidence points to a modulation of neutrophilic inflammation with minimal effect on eosinophil driven inflammation(168). This is surprising, as *in vitro* studies show that macrolides have significant effects on eosinophils and mediators such as IL-5, ECP, eotaxin, GM-CSF(161). Nevertheless, the presence of atopy and high IgE levels have been shown to reduce treatment efficacy(175, 176), however, the evidence for excluding such patients is weak and needs further verification. Macrolides have even been shown to reduce bone remodeling which may have consequences for patients with CRSwNP with regard to their ostiomeatal complex obstruction(177).

There is only one randomized clinical trial looking at the effect of macrolides in CRS, however patients with nasal polyposis were excluded(176). As such this limits its clinical applicability as it is in this group that macrolides are most effective. Moreover, the authors looked at patients when they were steroid naive. In clinical practice, intranasal steroids form the mainstay of treatment and as such it seems unlikely that macrolides will progress to becoming first-line agents for treating this condition.

Bronchial asthma is a comorbid condition of CRS and in some centres more than 50% patients have lower airway involvement(1). Around 10% patients with asthma are estimated to have nasal polyposis, the figure increasing to 40% in patients with aspirin sensitivity(99). There are no clinical trials looking at the effect of long term low-dose macrolide therapy on lower airway outcomes in chronic rhinosinusitis(178). Macrolides are known to have a variety of effects on the lung such as decrease in bronchial hyperresponsiveness(179), sputum eosinophil counts(180), decrease in nighttime symptoms and improvement in airway calibre(163). Finally, it is unclear as to how long this immunomodulatory effect lasts for, i.e. is there evidence of a more lasting disease modification than observed with steroids.

Aspirin intolerant patients

Prevention

Patients should be warned to avoid all drugs with COX-1 inhibitor activity. Although selective COX-2 inhibitors and paracetamol appear to be safe, the first dose should preferably be administered in hospital under monitoring. The role of avoiding preservatives, additives and high salicylate foods is controversial, with some benefit reported in open studies(9).

Leukotriene pathway modification

There is evidence that leukotriene modifiers like montelukast ameliorate nasal symptoms, decrease nasal response to an aspirin challenge and reduce the need for corticosteroids(181-183). In an uncontrolled prospective study of 678 patients with AIR, leukotriene modifiers alone or in combination blocked lower respiratory tract

reactions rather than upper respiratory symptoms during aspirin challenge in some patients. There was no change in the overall rate of positive challenge results(184). Leukotriene modifiers are only partially effective and patients often experience breakthrough nasal and bronchospasm despite treatment(185).

Aspirin desensitisation

Aspirin desensitisation is an important therapeutic option for patients who have inadequately controlled AIR and/or asthma despite treatment with topical corticosteroids and leukotriene-modifying drugs. Aspirin desensitisation reduces the reactions to aspirin by repeated and increasing exposure to prudent daily doses until all reactions cease. Just like provocation testing, desensitisation can be performed via the oral, endonasal or inhalational routes.

Aspirin desensitisation has been described as a treatment modality since 1922(186). In 1977, Bianco et al tried the bronchial provocation test with L-ASA in asthmatic patients intolerant to aspirin, and observed the existence of a refractory period post-challenge(187). At the end of the trial (within 20 days), all patients tolerated 500 mg of aspirin by mouth. Several author groups have since reported successful desensitisation with good clinical efficacy, a low risk-profile and high cost-effectiveness(188). There is some evidence that desensitisation may be more effective for rhinosinusitis symptoms than for lower airway symptoms, but overall patient hospitalisations and emergency department visits are reduced(189).

A typical oral regime will consist of gradually increasing doses of oral aspirin, and desensitisation takes a few days to achieve. Different authors have recommended various regimes and dosages ranging from 100 mg to 1300 mg of oral aspirin so as to

maintain a reduction of polyp size, improvement in olfaction and need for revision surgery, and improvement in lower airway outcomes(190-192). The European Network of Aspirin-Induced Asthma (AIANE) have described these procedures used to obtain desensitisation as 'adaptive deactivation' to distinguish them from the desensitisation obtained by specific immunotherapy in allergic asthma(94). 'Rush deactivation' is obtained in two days by administering L-ASA until induction of tolerance. 'Individual dose titration' is a combination of inhalation and oral administration of aspirin, and complete tolerance (which means that a single dose of 500 mg of aspirin is safely tolerated) is generally induced in 12 days.

Endonasal desensitisation has also been successfully carried out using the topical application of L-ASA for the specific treatment of AIR(193, 194). One author group have reported a lack of efficacy with a regimen of 16 mg of topical L-ASA or placebo instilled intranasally every 48 hours for six months(195). However, the same group reported improvements in polyp scores, peak nasal inspiratory flow rates and nasal nitric oxide levels using an endonasal regimen of 30 mg/ml L-ASA to each side in a dose ramp from 2 mg/day, increased every two or three days up to a maximum of 54 mg/day(196). Endonasal desensitisation may be sensitive to local factors such as nasal polyp size, mucociliary clearance, or to the concentration or regime of L-ASA.

Initial desensitisation by any route must be followed by maintenance dosing, as this deactivated state is only sustained for two to five days after cessation of aspirin.

After this, one can ingest aspirin or NSAIDs on an indefinite basis by taking a maintenance dose of aspirin without any serious adverse effects in many cases(197).

With appropriate monitoring equipment and adequately trained personnel, this can

be done as an outpatient regimen(198). It is important to note that if doses of aspirin are missed for any reason for more than 48 hours, then a repeat graded desensitisation will need to be done, or else severe reactions may be provoked.

Inpatient desensitisation has been recommended for patients with the following risk factors: beta-blocker use, recent myocardial infarction, severe asthma, history of severe or life threatening aspirin or NSAID reaction, or any medical condition or drug treatment regimen that would make the management of severe asthma or anaphylactoid reaction difficult(198). Pre-treatment with a cysteinyl leukotriene modifier such as montelukast, zileuton (or both) significantly reduces the incidence and severity of any aspirin-induced bronchospasm(184).

With oral and inhaled desensitisation, there is a small but pertinent risk of anaphylactoid and bronchospasm. Full cardiopulmonary resuscitation equipment must be available in the centre conducting desensitisation, and the patient must be under the supervision of a practitioner with advanced life-support training for two to three hours. The risks of desensitisation also include cutaneous reactions and gastrointestinal symptoms (dyspepsia, gastritis or haemorrhage). These are observed in about 20% of patients treated with aspirin(1, 189). There should be a protocol for dealing with aspirin-induced reactions; for example, ocular reactions which can be treated with topical antihistamines, gastrointestinal symptoms with proton pump inhibitors or H2 blockers, urticaria/angioedema with adrenaline and steroids, and bronchospasm with inhaled beta agonists(96). Oral aspirin desensitisation followed by maintenance daily dosing may cause significant side effects, including gastrointestinal bleeding at high doses. There is some evidence to suggest that oral doses as low as 100 mg daily may be effective for maintenance therapy and this

could potentially circumvent some of the adverse effects associated with currently recommended doses of 300 mg(101).

Other medical treatments

Antimycotics and their lack of efficacy have been discussed in this thesis previously in the section on microorganisms in the pathogenesis of CRS. There is good evidence for the use of nasal saline lavage as an adjunctive therapy in CRS, with a good safety and economic profile(1, 199). There is also some evidence for the addition of mupirocin to the lavage solution for extensive crusting(200).

Antihistamines cannot be currently routinely recommended in CRS alone, but may have a role in concomitant AR in a steroid-sparing capacity(1). The issues with nasal sympathomimetic decongestants have been dealt with earlier and will be covered in the specific chapters.

FOCUS OF THIS THESIS

Based on the literature review so far, several foci worthy of further exploration have been identified. I propose:

1. To evaluate the role of the ostiomeatal complex in CRSwNP and to construct a non-invasive outcome measure that is portable, cheap and reproducible. We propose therefore to measure the effect of systemic steroid therapy on the paranasal sinus ostiomeatal complex in CRS using humming nasal nitric oxide as a surrogate endpoint, with a custom built nasal mask adapter.
2. To assess if an initial burst of oral steroid therapy can augment and maintain long-term intranasal steroid efficacy in chronic rhinosinusitis with nasal

- polyposis using a powered randomized double-blind placebo controlled parallel trial design in patients with moderate to severe CRSwNP. Critically, for the first time in the literature to assess a panoply of safety parameters including adrenal suppression and bone turnover in the medium to long-term
3. To evaluate in our cohort of patients with CRS what the prevalence of an aspirin-intolerant endotype was, using a nasal lysine-aspirin challenge, and to assess if it predicted a more severe phenotype of CRS i.e. assess if the endotype informed the phenotype so as to plan future trials in refractory patients and consider desensitisation.
 4. To optimise and extend the duration of use of nasal sympathomimetic decongestants by evaluating tachyphylaxis of response to nasal oxymetazoline and its reversal by fluticasone. This was to assess a hitherto unexplored paradigm in rhinology by using a double-blind randomized placebo-controlled crossover design to (a) Establish if tachyphylaxis and rebound congestion occurs after use of oxymetazoline nasal spray (b) Demonstrate if reversal of effect can be achieved by intranasal fluticasone propionate and (c) Dissect out the relative $\alpha 1$ -/ $\alpha 2$ -adrenoceptor components of tachyphylaxis using the $\alpha 1$ -antagonist prazosin. I also aimed to construct a custom-made framework for using laser Doppler flowmetry in the human nose.
 5. To perform a comparative evaluation of nasal blood flow and airflow in the decongestant response to oxymetazoline in the human nose so as to place laser Doppler flowmetry in the context of known and commonly used airflow measures of disease and to guide future research and power calculations.

Chapter 2: Methods

An overview of methods common to all chapters is described in this section. Specific methodology relevant to each study has been described in the respective chapter.

Objective outcomes

Peak Nasal Inspiratory Flow Rate (PNIF)

PNIF was measured using an In-checkTM flow meter (Clement Clarke International Ltd, Harlow, UK). After blowing their nose, horizontal positioning of the meter and correct restoration of the reading to zero, participants inspired forcefully from residual volume to total lung capacity with their mouths closed. All measurements were made whilst in a sitting position with a good seal around a purpose built facemask. The best of three consecutive readings were recorded. Participants were trained how to use it properly at baseline prior to inclusion in the study and technique assessed and reviewed as indicated in the preceding visit plans. The participants were handed PNIF diaries to record at home if required in the study. Recordings were made prior to the morning dose of the investigational medicinal product and once again before the evening dose. The best of 3 readings were taken with the 2 highest within 10 L/min of each other.

Holmstrom and coworkers showed a highly significant correlation between PNIF and nasal airways resistance before and after allergen provocation testing(201). More recently, PNIF has been shown to correlate with nasal symptoms in patients with CRSwNP(202, 203). Peak nasal inspiratory flow has been shown to be more sensitive than acoustic rhinometry or rhinomanometry in evaluating nasal responses to provocation testing and correlates well with mucosal changes(204, 205). Furthermore it is portable, easy to perform and independent to the effects of the nasal cycle; the most common reason for false-positive results in nasal patency studies(206). Moreover, normative values have now been published for an adult

Caucasian population, allowing for assessment of deviation from standardised values and standard deviation in the subject populations(207).

Nasal mucosal blood flow using Laser Doppler Flowmetry

Nasal blood flow was measured using single point laser Doppler flowmetry (LDF)(208). Laser Doppler Flowmetry is a well-established technique for the non-invasive evaluation of microcirculation in man(209). A custom built PF-5 needle probe attached to the MBF3D™ monitoring system (Moor Instruments, UK) to estimate mucosal blood flow(210).

This probe has a right-angled tip with a red or near-infrared low power Helium-Neon laser light emitting diode which uses the coherent properties of laser light to monitor the mean velocity and density of the moving red blood cells in a unit of tissue(210). The light that is scattered back off moving red blood cells, undergoes Doppler shifts in frequencies, the amplitude of which is proportional to the velocity and density of red cells. The major advantage over other techniques such as ¹³³Xenon clearance, hydrogen clearance or photoplethysmography is that it gives a continuous signal that is responsive to subtle changes in vascular fluctuation(211). In the nose, Olsson and coworkers have shown that up to a distance of 3.5 mm between the probe and the mucosa, there is no change in the output signal(212). The reproducibility using the coefficient of variation with laser Doppler flowmetry is purported to be around 10%(213).

The depth of tissue penetration varies according to the optical properties of the tissue e.g. 1-2 mm for the skin and up to 6 mm for the gastrointestinal tract(208).

Moreover, in the nasal mucosa, Laser Doppler Flowmetry tends to measure the more superficial mucosal blood flow as opposed to other techniques such as Xe¹³³.

However, since its depth of penetration is unknown in the nasal mucosa, this is not proved beyond doubt. For example in the skin microcirculation of the fingernail bed, laser Doppler flowmetry was found to reflect flow in other vessels than simply the superficial capillaries(214). Thus it is entirely feasible, that in the nose, which has a similar extensive array of arteriovenous shunts just underneath the superficial mucosa, laser Doppler flowmetry (LDF) measures more than just the superficial capillary flow i.e. also captures partly or in total, the arteriovenous shunt flow. The Moor® instrument model has been shown to have higher signal to noise ratio than the Periflux® model(208).

In our study, the probe was positioned gently on the nasal mucosa of the inferior turbinate (the position marked and used at each subsequent visit) using a 2.7 mm 30° endoscope (Karl Storz-Endoskope; Tuttlingen, Germany). Horizontal head position and immobilisation was achieved with a custom-made head strap and chin-stabilizer. Olsson and coworkers have used a fixed skull-cap in a supine position to measure nasal blood flow using LDF(212). We decided to use an upright position using a custom built stable support system with a strap for the head as demonstrated in the figure. We felt this was more likely to be tolerated by patients on repeated visits and allowed us to visualize the nasal mucosa using the endoscope and administer medications easily. A further modification we made was there was a parallel holder for the nasal rigid endoscope along with the laser probe, which meant that we could always view the tip of the probe as it entered the nares. This ensures that our subjects felt comfortable during this procedure. A micromanipulator with an x-y-z axis

translator was used for fine adjustment of probe position. An average of 5 minutes of reading was taken after an initial stable tracing of 3 minutes was achieved.

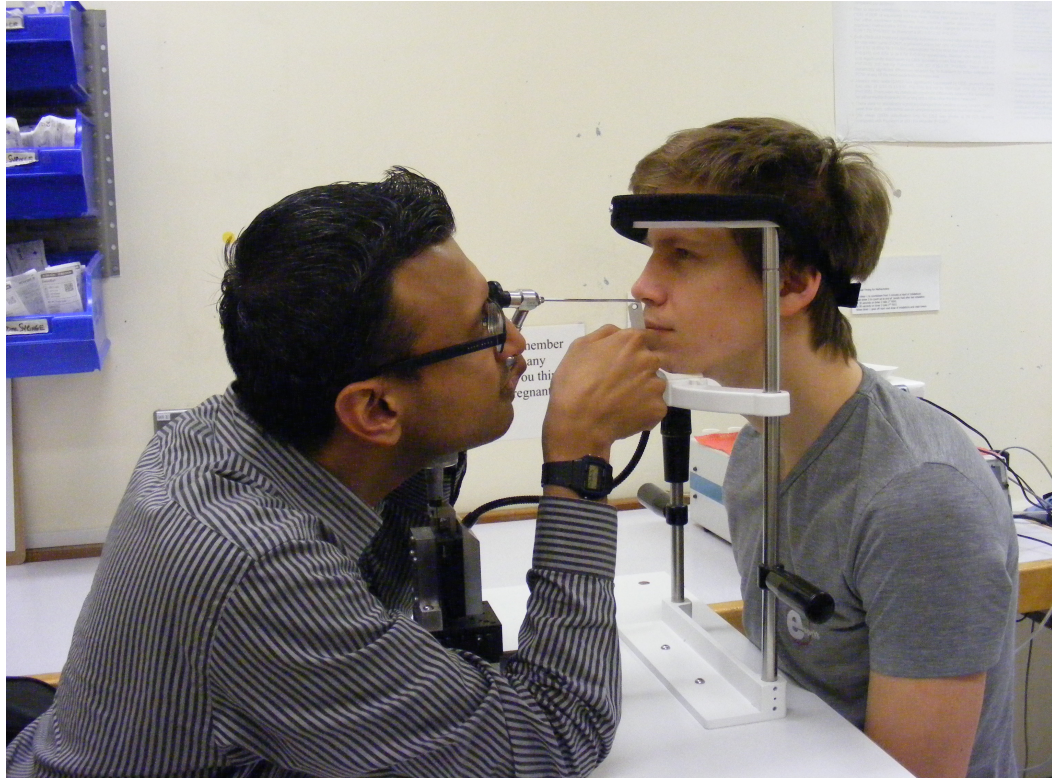


Figure 3. Custom built table framework for laser Doppler flowmetry with PF-5 probe 1 of 2.

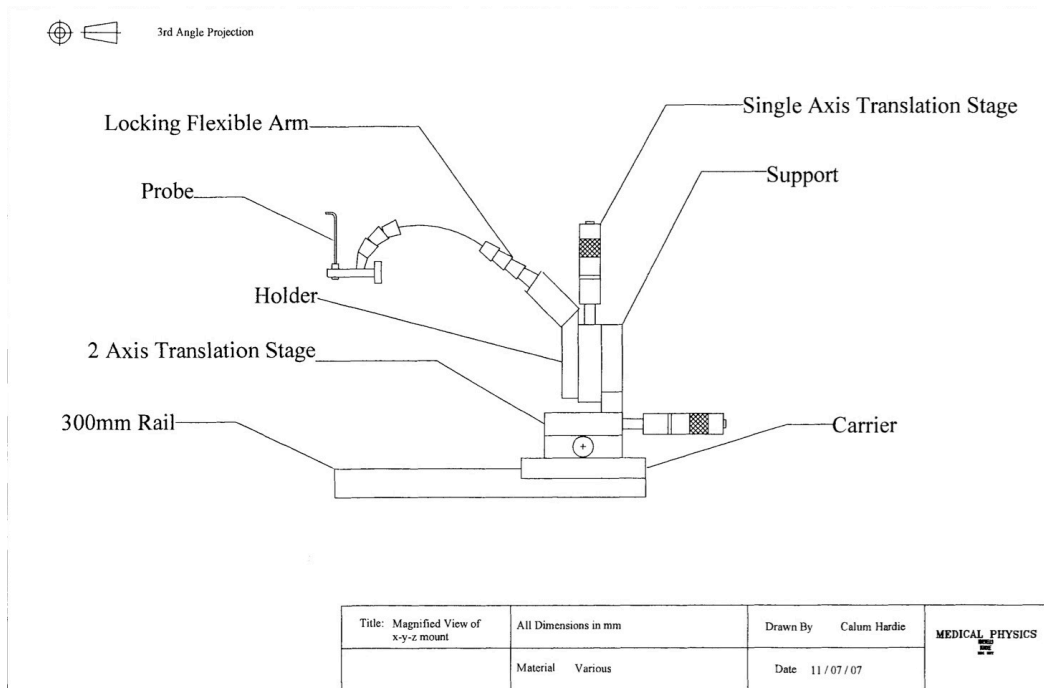


Figure 4. Custom built table framework for laser Doppler flowmetry 2 of 2.

Nasal endoscopy

Nasoendoscopy was performed using a 2.7 mm 30° endoscope (Karl Storz-Endoskope; Tuttlingen, Germany) with an integrated endoscopy camera system (LCH 01-D, Xion Medical, Berlin, Germany) and where required, standard video sequences were stored on a computer. These were later independently reviewed in a blinded fashion as per the study protocol. Where present, nasal polyps were graded using the system of Lildholdt et al.⁽⁸²⁸⁾: the degree of nasal polyps was classified in relation to the turbinates in four steps (0 – 3), where 0 is no polyposis, 1 is ‘mild’ polyposis (small polyps not reaching the upper edge of the inferior turbinate), 2 is ‘moderate’ polyposis (medium polyps between the upper and lower edges of the inferior turbinate) and 3 is ‘severe’ polyposis (large polyps reaching the lower edge of the inferior turbinate).

Sensitivity testing

Skin prick allergy sensitization testing was used for establishing sensitization as an inclusion/exclusion criteria or for characterization of atopic status. Standardized skin prick testing has been shown to be highly precise and reproducible and in the presence of a right symptomatology supports the diagnosis of allergic rhinosinusitis(215-217). Subjects were asked to withhold antihistamines and leukotriene receptor antagonists for at least 4 days prior to the test. Allergen drops (Bencard Testing Solutions: Welwyn Garden City, UK) were applied to the forearm; they were then pressed into the skin using disposable lancets. A standard protocol was used; the forearm was then marked using an adhesive strip of numbered tape, with markers 2cm apart. A positive response was defined as any wheal with a diameter that was 3mm greater than the negative control, at least 15 minutes after skin prick. A positive (histamine) control was used to exclude false negatives due to medication use, and a negative (excipient) control used to exclude false positives due to intrinsic skin hypersensitivity or diluent sensitization. Since the correlation of wheal measurements with allergic disease activity is poor, the test was not used in a quantitative manner i.e. reactions were recorded as present or absent.

Serum total IgE levels were measured in some studies using radio-immunoassays or enzyme immunoassays(218). Adult total serum IgE levels above 100 to 150 KU · L⁻¹ are considered above normal, but the finding is non-specific, associated with other allergic, parasitic and unrelated diseases. Further cytological or histological markers of disease inflammation or surrogates of the allergic process may be assessed using mucus brushings or scrapings or even biopsies(209). More recently, there has been

an increased interest in serum/nasal EDN as a marker of eosinophils as the major effector cells (as opposed to the Th2 cells directing the process) of allergic inflammation(219).

Nasal scrapings are a pain-free and well established method of collecting cells from the nasal mucosa for looking at inflammatory processes(220). The Rhino-Pro nasal curette (Arlington Scientific, Inc., Utah, USA) was used for this purpose. Patients remain in the seated position. Adequate lighting in the form of a head-lamp and a Thudicum's nasal speculum was used to gently open the nares. Under direct visualisation a small scrape was taken from the middle of the inferior turbinate. Briefly, after scraping, the curette was immersed in a 10-ml tube containing phosphate-buffered saline; the fluid recovered was centrifuged at 220 g per min for 10 min, and the pellet resuspended in PBS (2 ml). Cell suspensions were filtered to reduce the quantity of mucus, and cytopsin slides were prepared. Samples were stained with Diff Quik stain and analyzed using an optic microscope. The number of inflammatory cells was expressed as a mean of 10 fields at 100 times magnification. An open cell Polyurethane Foam Sampler (28 x 18 x 6 mm) with a fluid retention capacity of ~2.5 ml or an IVALON® 4000 (Ivalon surgical products, San Diego, USA) sinus pack was placed in the nasal floor posterior to the mucocutaneous junction for 10 minutes as described in literature(209). The secretions were immediately placed on ice. Following removal, cellular elements were separated from the liquid phase by centrifugation at 500xg for 5 minutes at 4°C. The cell-free supernatants were homogenized by ultrasonification at 160 watts for 5 minutes. The amount of liquid is measured volumetrically. Then aliquots of 100 µL are stored at –20°C for further processing. Nasal secretions were diluted 1:10 and cytokines analysed on a Bio-Plex™ Suspension Array System (Bio-Rad Laboratories, Hemel

Hempstead, UK) for a variety of Th1 and Th2 cytokines including IL-5, IL-8 and GM-CSF. We also measured IgE to *Staphylococcus aureus* enterotoxins (A, B, TSST-1), IgG (1-4 isotypes) to *Aspergillus* and *Alternaria* species with the fluorescence immunoassay (ImmunoCAP 250, Phadia Ltd., Milton Keynes, UK). Nasal polyp biopsy was taken under topical anaesthesia in the form of Lignocaine Hydrochloride. It is a painless procedure commonly performed in the outpatient clinic. Nasal tissue specimens were weighed, and 1 mL of 0.9% NaCl solution added for every 0.1g of tissue. The tissue was homogenized with a mechanical homogenizer at 1811 x g for 5 minutes on ice. After homogenization, the suspension was centrifuged at 3000 rpm for 10 minutes at 4°C and the supernatants separated and stored at –80°C until analysis(43).

Acoustic Rhinometry

Acoustic rhinometry was measured using an AI Executive acoustic rhinometer (GM Instruments, Ashgrove, Kilwinning, UK) according to the consensus report on acoustic rhinometry and Rhinomanometry issued by the Standardisation Committee on Objective Assessment of the Nasal Airway, I.R.S. and E.R.S.(221). A probe was inserted 0.5cm into each nostril to obtain a sufficient acoustic seal without distorting the nasal anatomy. Participants will hold their breath during the procedure and a probe-stand was used in order to ensure correct positioning of the probe. Correct environmental controlling will be done as outlined in the departmental standard operating procedure manual, which is in concordance with the European guidelines. For mucosal changes the volume 2-5cm seems to be an important variable and measurements were made of the minimal cross-sectional area (MCA) at the nasal valve (approximately 2cm from the nasal orifice) as outlined in a previous

study.(204) The total minimum area was taken to be sum of the measurement from the right and left nostrils.

Active Anterior Rhinomanometry

Anterior rhinomanometry was performed according to the consensus report on acoustic rhinometry and Rhinomanometry issued by the Standardisation Committee on Objective Assessment of the Nasal Airway, I.R.S. and E.R.S.(221). This was used to calculate the total nasal airways resistance using the formula

$1 \div R = (1 \div R1) + (1 \div R2)$. NAR was measured at 150 Pa and tidal breathing using an NR6 rhinomanometer (GM instruments, Kilwinning, UK). In subjects free from signs of nasal disease, mean total resistance has been reported to be around 0.23 Pa cm⁻³s⁻¹, ranging between 0.15 and 0.39 Pa cm⁻³s⁻¹(222). Reproducibility of rhinomanometry is variable in the literature and depends on the machine, variation related to subject technique, and variation caused by changes in airway size and shape because of individual differences, and requires serial measurements to be truly useful. Jones and coworkers have found a high correlation between NAR, as measured by anterior rhinomanometry and PNIF and also subjective nasal congestion(202).

Nasal Nitric Oxide

Nasal nitric oxide was measured at the same time of the day using a chemoilluminescence analyzer (NIOXTM, Aerocrine AB, Stockholm, Sweden) under standard conditions using one of three techniques: First, the standard aspiration technique recommended by the ATS/ERS guidelines(223) using a unilateral nasal olive, breathholding and velum closure at a flow rate of 0.05 L.s⁻¹; Second, single-

breath exhalation without phonation (quiet measurement) with a tight fitting nasal mask at a flow rate of 0.2 L.s^{-1} with a sustained plateau of at least 8 seconds and mean values calculated as average of last 70% of exhalation(224); Third, patients exhaled as described above but with nasal humming for at least 6 seconds. Patients were trained to hum at frequencies close to a 128Hz tuning fork with the mouth closed and given at least 3 satisfactory trials prior to the final measurements(225). Three technically adequate measurements were made with each technique. Each humming manoeuvre was separated by 5 minutes intervals for the sinus NO to recover(225). Maniscalco and coworkers also validated the handheld MINO ® (Aerocrine AB, Stockholm, Sweden) electrochemical analyser against a more standard chemoluminescence analyzer (NOA, Sievers) and demonstrated good agreement between the two techniques in 30 subjects (15 rhinitis and 15 healthy volunteers)(226). Given that the supra-velar airway can generate nitric oxide in several parts per million as opposed to parts per billion in the lower airways, measurement techniques are yet to be fully standardized and nasal NO remains a strictly research tool so far(223). It is yet to be fully understood how much the nasal mucosa contributes to the NO production vis-à-vis the paranasal sinuses. While it is possible that in CRS resulted impairment of the ciliated epithelium of the paranasal sinuses, which reduces its normal ability to express iNOS and produce NO decreases nNO levels, the more likely explanation is that the dominant factor in nasal NO measurements is the paranasal sinus NO(227, 228). What this means is that, rather than a measure of nasal mucosal inflammation as thought until recently, it is likely that nasal NO is a surrogate of the patency of the ostiomeatal complex(229). Boot and coworkers established the reproducibility of nNO with a coefficient of variation (CV) at day 1 of 16% and day 7 of 21%(209).

Subjective outcomes

Total Nasal Symptoms score (4-point scale)

The TNS-4 score is the sum of scores for nasal run, blockage, itch and sneeze, each measured on an ordinal scale of 0, 1, 2 or 3 representing no symptoms, mild, moderate or severe symptoms respectively. This results in an integer score for global TNS of 0 to 12. We have published minimal important differences for this score previously as 0.55 units(230).

Mini Rhinoconjunctivitis Quality of Life Questionnaire

The Mini RQLQ(231) is a self completed, rhinoconjunctivitis specific quality of life questionnaire. It has 14 items across 5 domains (activities, practical problems, nose, eye and other symptoms). Each item is scored for the preceding week on a scale from 0 (not troubled) to 6 (extremely troubled). The overall (and domain) scores are the mean of all (all domain) scores. The mini-RQLQ retains a strong association with the RQLQ ($r=0.91$), and has been shown to be more reliable (interclass correlation coefficient [ICC] 0.93), and responsive to change. It also has good construct validity (correlation with symptoms scores, the SF-36, a feeling thermometer and standard gamble(232, 233)). The MID has been published for this outcome and although this isn't a strictly CRS related outcome, the extensive use in the rhinitis literature and construct and content validity in the variety of patient populations makes it a suitable patient reported outcome measure. The only caveat is that olfactory outcomes must be assessed separately similar to the SNOT-20(234).

Sino-nasal Outcomes Test – 20

This easy to administer disease specific questionnaire was devised after modification of the cumbersome RSOM-31(235). Participants are asked to score a list of 20

symptoms and social and emotional parameters. They are also requested to indicate the 5 most important items on the score sheet. Thus a total score and a domainal score is obtained. The list include the need to blow the nose, sneezing, runny nose, cough, postnasal discharge, thick nasal discharge, ear fullness, dizziness, ear pain, facial pain/pressure, difficulty falling asleep, waking up at night, lack of a good night's sleep, waking up tired, fatigue, reduced productivity, reduced concentration, frustrated, restless/irritable and being sad and embarrassed. The possible range is 0 to 5, with a higher score indicating a greater rhinosinusitis-related health burden. This tool role is gaining increasing approval with robust psychometric and clinimetric reliability, validity and reasonable responsiveness(236). However, many agree that the omission of nasal congestion and hyposmia limits the utility of this measure and in the National Comparative Audit of Surgery for Nasal Polyposis and Chronic Rhinosinusitis conducted by the Royal College of Surgeons of England a modified SNOT-22 was used(142).

Visual Analogue Scale Scoring

This global score of disease severity is validated in the adult population with CRS(1). The disease can be divided into mild, moderate and severe based on a total severity scale score of 10cm as follows: mild = 0-3, moderate = 4-7 and severe = 8-10. To evaluate the total severity, the patient is asked to indicate on a VAS the answer to the question: How troublesome are your symptoms of rhinosinusitis? With minimum and maximum anchors as 'not troublesome at all' and 'worst thinkable troublesome'. A VAS > 5 affects the patient quality of life.

Olfactory testing

In a multicentre study across 10 European countries Szczeklik and coworkers evaluated the natural history of CRS and found that up to 55% of patients had an impaired sense of smell(35). This is particularly important, as hyposmia constitutes a primary symptom of CRSwNP and has a significant impact on quality of life(1, 237, 238). CRSwNP causes olfactory impairment due to mechanical obstruction, sensorineural defects secondary to mucosal inflammation and stem cell loss(239).

When treated with topical steroids alone, improvement in the patient's sense of smell often 'lags behind' the improvement in nasal obstruction(240), possibly due to limited access to the olfactory cleft. Thus assessment of olfaction is critical in the complete assessment of CRSwNP. As mentioned previously, validated quality of life tools such as SNOT-20 lack a domain for the assessment of the sense of smell(236).

Although SNOT-22 has been designed to overcome this and used in large-scale cross-sectional observational studies(142), it is not validated in CRS and its responsiveness to treatment is unknown.

The University of Pennsylvania Smell Identification Test (UPSIT) is a 40-item validated self-administered test with a test-retest reliability of 0.94(241). It has normative values based on over 4,000 persons. The categories of dysfunction include mild, moderate, severe and total. It has an inherent ability to detect malingering.

Unfortunately, some smells are not relevant to the UK (e.g. skunk), the test is expensive to administer and is time consuming. Hence we chose to use a combination of the Pocket Smell Test® which is a 3-item, forced choice, short derivative of the UPSIT® to assess olfaction in our study (Sensonics, Inc. New Jersey, United States)(242) along with a 10-point visual analogue scale(1, 6). This test has been validated in neurological disorders and has been used to validate other short olfaction testing(243, 244).

Safety Outcomes

There are remarkably little medium to long-term controlled data in rhinology with regards to systemic safety data (when compared to efficacy data) in adults. This is surprising, given the rampant use of highly lipophilic drugs, such as intranasal corticosteroids, when measuring the concentration in the water soluble plasma compartment is only looking at a relatively small portion of the systemically available drug, which has a large total volume of distribution(245). Not only will this underestimate the total body exposure, but also ignore the prolonged elimination half-life with systemic tissues acting as slow-release reservoirs. This may lead to an apparent discrepancy in bioavailability after a single dose and systemic bioactivity at steady-state. Thus studies measuring plasma concentrations after single doses for intranasal corticosteroids may underestimate the potential for systemic adverse effects in the steady state(245). Hypothalamic-pituitary-adrenal axis activity has been shown to be one of the most sensitive markers of systemic bioactivity(155). Indeed, the measurement of overnight urinary cortisol-creatinine excretion has been shown to be as sensitive as an integrated 24-hour plasma or urinary free cortisol collection and is more sensitive than a spot measurement of 8 a.m. plasma cortisol levels(246, 247).

Overnight urinary cortisol: creatinine ratio

At 22:00 hours on the night before each visit, subjects emptied their bladders and then collected all urine produced until 08:00 hours the following morning. Cortisol (Diastorin, Wokingham, UK) and creatinine (Cobas-Bio, Roche Diagnostics, Basel, Switzerland) were assayed and ratios calculated to determine the overnight urinary

cortisol: creatinine ratio (OUCC), a sensitive marker of drug effects on the HPA axis(246). The coefficients of variation within and between assays were 9% and 6.2% respectively, for cortisol; and 2.9% and 4.6% for creatinine. Each participant's samples were processed within a single assay. The assay has no cross reactivity with fluticasone propionate.

Similarly, there are no long-term controlled data in the literature evaluating markers of bone turnover. The bone mass at any given time of life represents a complex interplay of genetic loading and other risk factors such as age, ethnic origin, sex, body size, diet, use of alcohol and tobacco, physical activity, thyroid status, and sex hormone status(248).

Chapter 3 is the first and in our knowledge the only randomized clinical trial to include a comprehensive safety data profile within its portfolio.

Chapter 3: Re-establishing ostiomeatal complex drainage: a prospective study

Aims: To evaluate the effect of systemic steroid therapy on the sinus ostiomeatal complex in chronic rhinosinusitis using humming nasal nitric oxide as a surrogate endpoint.

Introduction

Chronic rhinosinusitis with nasal polyposis (CRSwNP) is a common chronic inflammatory disorder with significant morbidity, major healthcare costs and a high socioeconomic burden(26). The course of CRSwNP is characterized by multiple relapses and short courses of oral steroids are frequently used as rescue therapy to relieve inflammation, reduce polyp size and improve physiologic sinus drainage. A fundamental role in the pathogenesis and perpetuation of CRSwNP is attributed to blockage of the paranasal sinus outflow tracts due to inflammation and eventually hyperostosis. However, it is often difficult to determine clinically if systemic steroids improve sinus ostial patency, and if this occurs concurrently with other outcomes such as endoscopic polyp score, symptoms and nasal airflow.

Nitric oxide (NO) is an important regulatory molecule and is produced in the respiratory tract by a variety of sources such as endothelium, epithelial cells, smooth muscle and inflammatory cells, with highest measured concentrations in the paranasal sinuses(249). NO is a sensitive non-invasive marker of inflammation and ciliary dysfunction and has played an increasingly important role in lower airway conditions such as asthma; however its niche in the diagnosis and management of the upper airway has not been firmly established. Moreover, in patients with CRSwNP despite higher levels of inflammation compared to uncomplicated allergic rhinitis, paradoxically low levels of nasal NO have been demonstrated due to sinus ostial obstruction and perhaps contributed to by mucociliary dysfunction(229). Conversely, following medical or surgical treatment for CRSwNP levels of nasal NO have been shown to increase significantly(250, 251).

Having the patient hum at optimal frequencies with the mouth closed during expiration has been shown to induce a large increase in nasal nitric oxide levels, and

it has been proposed that this reflects rapid gas exchange between the nose and paranasal sinuses i.e. sinus ostial patency(252). To our knowledge, there are no studies in the literature evaluating the effect of systemic steroid therapy on the humming fraction of nasal nitric oxide in nasal polyposis. This is crucial, as NO measurement may fill a niche in patient management that radiography, including CT imaging, cannot due to cost and radiation exposure. Moreover, NO measurements are quick, non-invasive, harmless and can be done through portable chemoilluminescence analyzers(226). Additionally, humming NO as opposed to other methods of measuring NO may be more sensitive at detecting a treatment response as it may boost the signal for detection particularly in severely obstructed patients.

We hypothesized that a short course of oral steroids would increase the humming fraction of nasal nitric oxide as compared to baseline values as a surrogate of sinus ostial patency. We also aimed to evaluate if the humming NO is a stronger indicator of this increase than other methods of measuring NO, and if it correlates to improvements in clinical parameters for sinonasal disease.

Methods

Participants and Settings

Non-smoking, adult patients with CRSwNP, with or without asthma and a nasoendoscopic polyp grade (2.7 mm 30° endoscope Karl Storz-Endoskope; Tuttlingen, Germany) of 2 or above as deemed by the Lildholdt grading system(253), and normal serum aspergillus IgE and IgG levels were included. Patients were excluded with known aspirin sensitivity, systemic steroid treatment during the prior three months, sinus surgery within 1 year, acute upper respiratory tract infection within 1 month, antibiotics taken within 1 month, nasal airway obstruction due to septal deviation >50%, pregnancy and lactation were excluded. These patients are seen in our joint surgical and medical rhinology clinic and are routinely treated with a 2-week course of oral prednisolone 25mg/day followed by intranasal corticosteroids as part of their routine clinical care. In our clinic, we also regularly measure peak nasal inspiratory flow, quality of life and nitric oxide as part of routine practice, to which we added the measurement of humming NO. Institutional review board approval was therefore not necessary as the Tayside Committee for Medical Research Ethics gives standing approval as part of patients' routine clinical care. No alterations were made to their regular medications.

Measurements

Peak nasal inspiratory flow (PNIF)(204, 205, 230), Sinonasal Outcomes Test-20 (SNOT-20)(236), global visual analogue scale (VAS) score (answering the question “How troublesome are your overall symptoms of rhinosinusitis?” 0 being ‘Not Troublesome’ and 100 mm being ‘Worst Thinkable Troublesome’)(13), hyposmia VAS(6) (0 was ‘No Sense of Smell’ and 100 mm was ‘Excellent Sense of Smell’)

were measured before and after their routine treatment. Similarly, nasal nitric oxide was measured at the same time of the day using a chemoluminescence analyzer (NIOXTM, Aerocrine AB, Stockholm, Sweden) under standard conditions using three techniques: First, the standard aspiration technique recommended by the ATS/ERS guidelines(223) using a unilateral nasal olive, breathholding and velum closure at a flow rate of 0.05 L.s⁻¹; Second, single-breath exhalation without phonation (quiet measurement)with a tight fitting nasal mask at a flow rate of 0.2 L.s⁻¹ with a sustained plateau of at least 8 seconds and mean values calculated as average of last 70% of exhalation(224); Third, patients exhaled as described above but with nasal humming for at least 6 seconds. Patients were trained to hum at frequencies close to a 128Hz tuning fork with the mouth closed and given at least 3 satisfactory trials prior to the final measurements(225). Three technically adequate measurements were made with each technique. Each humming manoeuvre was separated by 5 minutes intervals for the sinus NO to recover(225).

Statistical Analysis

Ours was a prospective cohort study with a follow-up duration of two weeks. Each outcome was assessed for normality using the Shapiro-Wilk test and by visual inspection of histograms and Q-Q plots, with consideration of previous datasets and literature. Non-Gaussian data (Nitric oxide) were log-transformed prior to analysis to normalize the distribution. A correction for exhaled lower respiratory nitric oxide levels was applied by subtracting respective tidal nitric oxide levels(254). Outcome comparisons before and after treatment were made using paired Student's t-tests and mean differences (or geometric mean fold change for logtransformed variables) with a significance level set at $P < 0.05$. Secondary analyses included area under the curve for exhaled and humming NO and standardized response means (SRM) for all three

methods of measuring NO. The area under the curve (AUC) for quietly exhaled nitric oxide and humming nitric oxide were calculated at time-points 0, 2, 4 and 6 seconds from the start of exhalation using a linear trapezoidal rule. AUC comparisons were made pre and post-treatment and are more sensitive than average measurements. SRM is defined as the ratio of the mean change in scores to the standard deviation of the change and is a relative measure of effect size and responsiveness. It allows the expression of the signal of change in an outcome relative to its variability(255). Lastly, a correlational analysis was performed comparing the three methods for NO estimation with all other outcomes and Pearson correlation coefficient was calculated.

All analyses were performed with SPSS version 15, Chicago, IL, USA.

Abbreviations: ICS, inhaled corticosteroids as µg of CFC-beclometasone equivalent units; FEV₁ %, forced expiratory volume in 1 second % of predicted value; S, intranasal corticosteroids; L, leukotriene modifiers; A, antihistamines; BUD, Budesonide; FP, Fluticasone propionate; BDP, Beclometasone dipropionate

Effects of oral prednisolone on all outcomes is shown in the following Table 2 and Figures 2, 3, and 4.

Table 3: Outcomes pre and post oral steroid.

Variable	Pre-steroid (95% CI)	Post-steroid (95% CI)	Mean difference (95% CI, P)
SNOT – 20 (units)	2.6 (2.1 to 3.1)	1.1 (0.7 to 1.4)	–1.57 (–2.03 to –1.10, <.0001)
Global VAS score (0- 100mm)	64.1 (51.6 to 76.8)	24.9 (12.9 to 36.9)	–39.2 (–48.9 to –29.6, <.0001)
Hyposmia VAS score(0- 100mm)	20.4 (11.0 to 29.8)	67.7 (5.7 to 80.8)	47.3 (31.3 to 63.4, <.0001)
Endoscopic polyp grading (0-6)	4.7 (4.0 to 5.3)	2.5 (1.5 to 3.5)	–2.2 (–2.8 to –1.5, <.0001)
PNIF (L.min ^{–1})	112.5 (78.1 to 146.9)	157.5 (132.1 to 182.9)	45.0 (33.3 to 56.6, <.0001)
Aspiration Nasal _{NO} (ppb)†	341.5 (254.2 to 458.7)	503.6 (376.2 to 674.3)	1.5 (1.1 to 1.9, 0.006)‡
Exhaled Nasal _{NO} (ppb)†	11.2 (5.9 to 21.1)	23.9 (17.1 to 33.3)	2.1 (1.2 to 3.9, 0.02)‡
Humming Nasal _{NO} (ppb)†	31.2 (15.6 to 62.8)	152.9 (91.7 to 255.1)	4.9 (2.2 to 10.7, 0.001)‡

Abbreviations: CI, confidence interval; SNOT, sino-nasal outcomes test; VAS, visual analogue scale; PNIF, peak nasal inspiratory flow rate; NO, nitric oxide. Nasal NO values shown are corrected for tidal NO levels and measurements were taken at a flow rate of 0.2L.s^{–1}.

* Data are presented as arithmetic mean (95% CI) unless otherwise indicated.

† Geometric mean (95% CI)

‡ Geometric mean fold ratios (95%CI).

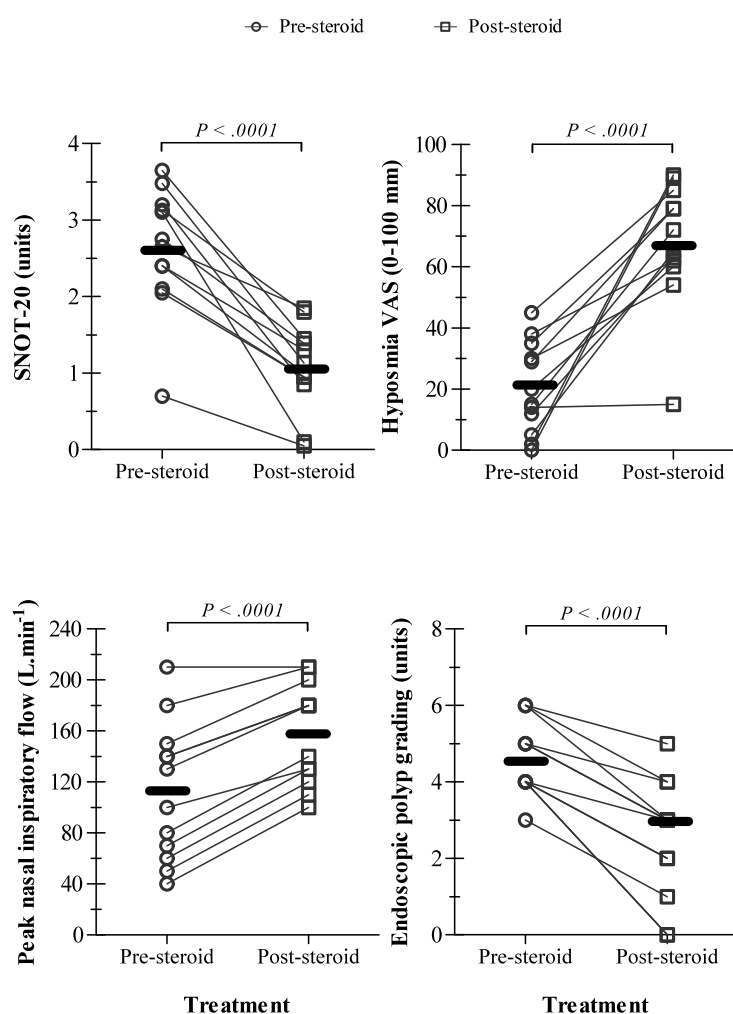


Figure 5: Outcomes pre and post oral steroids.

Figure 1. Scatter plots showing individual values for Sinonasal Outcomes Test 20 (SNOT-20), visual analogue scale for hyposmia (mm), peak nasal inspiratory flow rate ($L \cdot min^{-1}$) and endoscopic polyp grading before and after treatment with oral prednisolone 25mg per day for 2 weeks in patients with nasal polyposis. Horizontal bars represent arithmetic means. Treatment with systemic steroid resulted in global improvement in all subjective and objective outcome measures. P-value corresponds to the results of comparing means for pre- and post-treatment for each parameter.

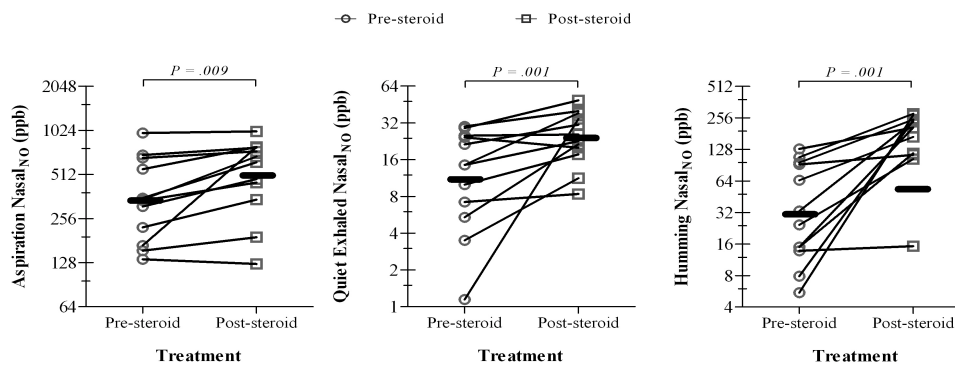


Figure 6: Nasal NO using different methods pre and post oral steroids.

Figure 3. Scatter plots showing individual values for nasal nitric oxide measured by aspiration, quiet exhalation and humming exhalation before and after treatment with oral prednisolone 25mg per day for 2 weeks in patients with nasal polyposis. Horizontal bars represent geometric means. Treatment with systemic steroid resulted in improvement in all three outcomes with the greatest improvement by the humming method. P-value corresponds to the results of comparing means for pre- and post-treatment for each parameter.

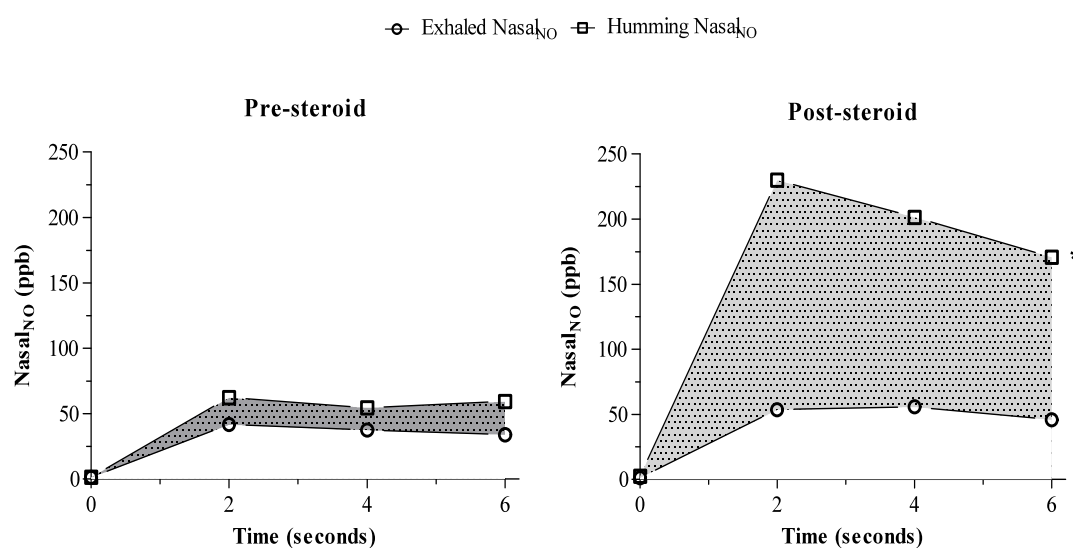


Figure 7: Time profile curve for exhaled and humming nasal NO pre and post oral steroids.

Figure 4. Time-profile for quietly exhaled fraction of nasal nitric oxide (NO) and humming fraction of nasal NO before and after treatment with oral prednisolone 25mg per day for 2 weeks in patients with nasal polyposis. Values denoted are arithmetic means and represent the total (uncorrected nasal and tidal values) exhaled and humming NO measured at a flow rate of $0.2\text{L}\cdot\text{s}^{-1}$.

* denotes significant difference ($P < 0.05$) between exhaled and humming fractions after (but not prior to) treatment with oral prednisolone 25mg per day for 2 weeks in patients with nasal polyposis.

In this study, SNOT-20 scores improved at 2 weeks i.e. post-steroid as compared to baseline by a mean difference (95% CI, *P*) of 1.6 (1.1 to 2.1, <.0001) units. The minimal important difference is 0.8 units making this clinically significant. Global VAS scores and hyposmia VAS scores improved by 39.2 (29.6 to 48.9, <.0001) mm and 47.3 (31.3 to 63.4, <.0001) mm respectively. PNIF increased by 45 (33.3 to 56.6, <.0001) L.min⁻¹ and endoscopic polyp score shrunk by a median (IQR) of 2 (1.75 to 2.25, <.0001) units.

Nasal NO measured by aspiration, exhaled and humming techniques increased post-treatment as compared to baseline as geometric mean fold ratio (95% CI, *P*): 1.5 (1.1 to 1.9, .009), 2.1 (1.2 to 3.9, .02) and 4.9 (2.2 to 10.7, .001), respectively.

The difference in the AUC of humming NO vs. quiet exhaled NO was mean difference (95% CI) 21.7 (–355.5 to 398.9) ppb.s⁻¹ at baseline and increased to 770.2 (393.3 to 1147) ppb.s⁻¹ post-steroid. The standardized response means were as follows for the three methods: aspiration = 0.97; exhalation = 1.05 and humming = 1.61.

The aspiration NO technique did not correlate significantly with any outcome. Quiet exhalation method showed significant correlation only with PNIF (*R* = 0.71, *P* = 0.01). Humming NO correlated significantly only with hyposmia VAS (*R* = 0.59, *P* = 0.04)

Discussion

In the present cohort study we have shown for the first time that treatment with oral prednisolone in patients with nasal polyposis results in an increase in the humming fraction of nasal NO (Figures 6,7). We have also shown that compared to the aspiration and expiration without humming techniques for measuring nasal NO, humming accentuated the difference in NO levels between pre- and post-treatment. Lastly, increases in NO levels with treatment occurred concomitantly with improvements in an array of clinical parameters including polyp size, symptoms and nasal airflow. Whilst it has been shown previously that oral steroids improve nasal symptoms, quality of life and sinus opacification scores on imaging, their effect on humming NO as a surrogate of sinus ostial patency has not been assessed(256). Ragab and coworkers showed that aggressive medical treatment for CRSwNP including initial oral steroids followed by topical steroids resulted in a significant increase in nasal NO over the 12 month follow-up period(144, 250). Delclaux and coworkers similarly showed an increase in nasal NO flux after treatment with oral and topical steroids and nasal washes(251). Until now, no one has evaluated humming NO as a surrogate of sinus ostial patency or compared it to other methods of NO estimation. Maniscalco and coworkers have demonstrated the absence of a nasal NO peak during humming in patients with CRSwNP with endoscopic findings suggestive of sinus ostial obstruction(257). Lundberg and coworkers have shown that in patients with CT scan findings of sinus ostial obstruction, there was an absent humming peak (vs. controls)(252). In the same paper, they claimed that humming NO peak levels recovered post surgery but these data were not shown. CT scans are considered crucial by many authors in the assessment of sinus ostial patency(6).

While CT may be valuable in the anatomical assessment of paranasal sinuses and their outflow tracts, they lack functional value in evaluating sinus ostia patency (e.g., an obstructed looking ostium on CT scan may be functionally patent or vice versa). High percentages of incidental radiologic findings are found in healthy individuals, normal nasal cycle may induce changes in the nasal mucosa, and radiological abnormalities correlate poorly with patient's symptoms and clinical outcomes(258). Moreover, in routine clinical practice in the UK, CT scans are not performed unless osseus/intracranial complications are suspected or as a staging/roadmap prior to surgery. Repeated CT scans cannot be justified in clinical practice due the high radiation risk, especially in nasal polyposis which has a remitting and relapsing course over many decades and may require many rescue courses of oral steroids - mean duration of symptoms in our study was 18.5 years (150). On the other hand, humming nasal NO is a quick and non-invasive technique to evaluate ostiomeatal patency(252). Indeed, recent advances in portable hand-held electrochemical analyzers have made it possible for these measurements to be made even in the community where most patients with CRSwNP are managed(1, 226). We believe that humming NO and CT scans provide complementary information assessing both functional and anatomical aspects of the disease respectively.

Ours is also the first study to evaluate the exhalation technique to measure nasal NO in medical therapy for CRSwNP. International guidelines currently recommend the aspiration method for measuring nasal NO(223). The nasal aspiration technique is advantageous with regard to velum closure and therefore not needing correction for tidal NO values. It is plausible that the correction for tidal NO is a factor influencing variability in our study. In a study by de Winter-de Groot and coworkers, the humming and breath holding methods showed the lowest variability and highest

short term and long term reproducibility with humming performing better than breath-holding(259). However, these findings were from studies conducted in healthy volunteers, patients with CRSwNP prefer exhalation technique due to the prolonged breath-holding with the former method, often accentuated by the fact that many of them have concomitant lower airway dysfunction. Moreover, we calculated the standardized response means (SRM) for all three nasal NO techniques as; aspiration method, 0.97; exhalation, 1.05 and humming, 1.61. SRMs are highly informative measures calculated by dividing the mean change in an outcome by the standard deviation of the difference, i.e. it is a measure of effect size or responsiveness. Because the denominator examines response variance, it provides a sensitive indication of ‘signal-to-noise’ ratio. It is readily apparent that humming nasal NO is the most responsive followed by exhalation and lastly aspiration.

We recognize the limitations of our study. Ours was an open label study in a small group of selected patients without a parallel control group. However, it represents real patients in actual clinical situations, and the global improvement in PNIF, quality of life, symptoms, olfaction and polyp scores (Table 2 and Figure 6) shows an adequate effect size. Interestingly, humming nasal NO correlated significantly with improvement in smell ($R = 0.59$, $P = 0.04$) and quiet exhalation correlated with PNIF ($R = 0.71$, $P = 0.01$). It is likely that the small numbers in our study reduced the statistical power to tease out further correlations. Even so, it is interesting that humming NO improves in parallel with one of the most disabling symptoms of CRSwNP i.e. the loss of sense of smell. Further larger controlled studies may be able to differentiate humming responders and non-responders and possibly see if an improvement in the sense of smell can be predicted based on baseline humming NO or change in NO after the initial oral steroid bolus. Humming NO may also be useful

in predicting which patients are candidates for long-term medical therapy without surgery. Conversely, lower humming NO could be used for early detection of relapse of disease or non-response to treatment. Humming NO may also be a useful objective investigational tool in future research trials evaluating sinusitis and allergy treatments in patients with CRS and may obviate the need for CT scans in some patients.

In summary, we have shown that treatment of patients with CRSwNP using prednisolone 25mg/d for 2 weeks is associated with an increase in NO levels utilizing the nasal humming during expiration technique. Compared to the aspiration and expiration without humming techniques for measuring nasal NO, the nasal humming technique accentuated the difference in NO levels between pre- and post-treatment, which implies that it is likely the most sensitive method for detecting improvements in sinus ostial patency. Lastly, increases in NO levels with treatment occurred concomitantly with decreases in nasal polyp size and symptoms (measured by the SNOT-20 questionnaire and the global and hyposmia visual analogue scales), and increases in peak nasal inspiratory flow. Further long term randomized studies are needed to evaluate the utility of humming nasal NO in the treatment algorithm of CRSwNP in the community during sequential step down from systemic to topical steroids, as well as postoperative improvement or relapse after endoscopic sinus surgery.

Chapter 4: To evaluate if initial oral
steroid therapy can augment and
maintain long-term intranasal
steroid efficacy in chronic
rhinosinusitis with nasal polyposis:
a randomized double-blind placebo
controlled trial

Introduction

Chronic rhinosinusitis (CRS) is a common chronic disorder in the developed world affecting 32 million people (16.3% of the population) in the United States and 15% of the European population(26). Annual healthcare costs for CRS have been conservatively estimated at \$6 billion, though this is likely to underestimate indirect costs due to lost productivity, sickness absenteeism and concomitant illnesses like asthma(26, 30, 34). Indeed, using the SF-36 questionnaire it has been shown that patients with CRS report lower quality of life scores than patients with congestive heart failure, angina, COPD and back pain(28). CRS and asthma may represent different aspects of the same inflammatory disease continuum and coexist frequently(260). Patients with concomitant CRS and asthma are more likely to have unstable lower airway disease with multiple exacerbations and a more severe phenotype of CRS(261). While the link between CRS and asthma has been investigated, its putative associations with chronic cough syndrome, depression and even lung malignancy are less well appreciated(30-33). CRS is diagnosed and managed by a wide variety of practitioners, including primary care physicians, otolaryngologists, pulmonologists and allergologists. It is one of the most common reasons for presenting to primary care and accounts for 21% of all adult antibiotic prescriptions in the US, despite a lack of evidence for efficacy(25, 26).

CRS with nasal polyposis (CRSwNP) is a distinct pathological subtype with a greater burden of symptoms and a higher relapse rate after treatment than those without polyposis(10). Intranasal corticosteroids are recommended as the treatment of choice for maintenance in CRSwNP(13). However, the typical course of the illness is marked by frequent relapses requiring either endoscopic sinus surgery or

rescue pulses of oral corticosteroids which are often referred to as ‘medical polypectomies’(9). We hypothesize this is partly attributable to the fact that topical therapies cannot effectively penetrate the ‘ostiomeatal complex’ (the outflow tract of the paranasal sinuses) and therefore fail to re-establish physiological sinus drainage. Surgical treatment temporarily relieves ostiomeatal complex blockage, but is not curative and serves primarily to facilitate penetration of topical steroid therapy i.e. establishing an access route. Moreover, with the potential for orbital and intracranial complications, surgery should be reserved for those who are refractory to maximal medical therapy(13). In our clinical experience, a short course of oral steroids, or ‘medical polypectomy’, improves long-term efficacy of topical therapy, however, there are no robust data to support this. Moreover, despite the high prevalence of concomitant asthma and use of inhaled corticosteroids, there are no data on long-term systemic steroid burden in these patients.

Both surgery and medical polypectomy are thought to improve sinus drainage via the ostiomeatal complex allowing better access to maintenance topical corticosteroids(262). Indeed, long-term therapy with intranasal corticosteroids alone leads to a steady loss of symptom control, in particular hyposmia, which is a key symptom in CRS(1, 150).

Current American and European management guidelines for CRSwNP do not acknowledge the need for a sequential approach encompassing induction and subsequent maintenance corticosteroid therapy(1, 6, 9, 30). Instead, existing treatment algorithms advocate the use of initial intranasal corticosteroid therapy, especially for primary care physicians and non-specialist practitioners, and recommend the use of oral corticosteroids only in refractory cases in secondary care(25). There is an unmet need to establish a standardised, long-term approach to

management, which maximises the benefits of corticosteroid therapy without undue adverse side effects. There are, however, no randomized clinical trials evaluating the medium to long-term efficacy of medical treatment regimes for CRSwNP. Little is known about treatment safety, in particular the effects on adrenal function and bone turnover in the medium to long-term. This is especially important, due to the high concomitant prevalence of asthma and concurrent therapy with inhaled corticosteroids. In addition, cellular steroid resistance has been reported in CRS; the illness being especially recalcitrant to therapy in patients with asthma and aspirin intolerance(101, 263). It is not known which local or systemic factors may predict response to treatment(264). Emerging evidence suggests a possible role for *staphylococcus aureus* enterotoxins (SAE)(50). These act as superantigens in the pathogenesis and disease modification of CRSwNP by orchestrating polyclonal IgE activation and stimulating eosinophil recruitment(13). These superantigens have also been shown to alter corticosteroid sensitivity and glucocorticoid receptor expression(265). There are no randomized clinical trials to examine the influence of systemic corticosteroids on multiclonal IgE activation induced by SAEs.

On this basis, we conducted the first randomized clinical trial evaluating the medium to long-term efficacy and safety of a treatment regime consisting of initial systemic induction with oral prednisolone followed by sequential maintenance therapy with intranasal corticosteroid as drops and spray. We hypothesized that the initial ‘medical polypectomy’ would provide greater and sustained improvement in nasal function, systemic inflammation and quality of life parameters in the medium to long-term after sequential step down to topical corticosteroids. We also evaluated a host of other clinical, molecular and histological factors as predictors of treatment response.

Methods

DESIGN OVERVIEW

We conducted a parallel randomized controlled trial of oral prednisolone versus placebo for an initial 2 weeks, followed in both groups by 2 months of fluticasone nasal drops followed by fluticasone nasal spray for 4 months for the treatment of CRS with NP.

SETTING AND PARTICIPANTS

Non-smoking adults with CRS with NP, with or without asthma, were recruited from a single-centre specialty clinic in Tayside, Scotland, where patients were referred for assessment by their primary care physician. Diagnosis of CRS with NP was made by ENT specialists according to the European Position Paper on Rhinosinusitis and Nasal Polyps 2007 criteria (13) and suitability was confirmed in each case as follows: On nasoendoscopy, participants were required to have bilateral nasal polyposis of grade 2 or above using the Lildholdt grading(266) and at least two of the following symptoms for >12 weeks; anterior and/or posterior nasal discharge, nasal obstruction and decreased sense of smell. Exclusion criteria included: treatment with oral corticosteroid within three months, sinus surgery within one year, recent upper respiratory tract infection, mechanical nasal airway obstruction due to septal deviation >50%, pregnancy and lactation. The study had Institutional Review Board approval from the Tayside Committee on Medical Research Ethics. All participants gave written informed consent.

RANDOMIZATION AND INTERVENTIONS

An independent off-site clinical trials pharmacist (Pharmacy Production Unit, Western Infirmary, Glasgow, United Kingdom) used a computer-generated random

allocation sequence to randomise the trial using block randomisation with block size of 4. The same pharmacist masked and blinded the prednisolone 25 mg tablet and an identical placebo tablet to double-blind the study from the investigator and participants. These were administered using sealed opaque envelopes at the research unit.

Following a screening visit, suitable subjects entered a two-week run-in, where treatment for CRS with NP was stopped. Participants were randomly allocated in a 1: 1 ratio to receive prednisolone 25mg/day or identical placebo for 2 weeks, followed by fluticasone propionate nasal drops (Flixonase® nasule, Allen & Hanburys Ltd, Uxbridge, Middlesex, United Kingdom) 400µg twice daily for 2 months and then fluticasone propionate nasal spray (Flixonase® nasal spray, Allen & Hanburys Ltd, Uxbridge, Middlesex, United Kingdom) 200µg twice daily for a further 4 months. From screening until the end of the study, no other rhinitis medications were permitted, including: antihistamines, leukotriene receptor antagonists, intranasal corticosteroids, and nasal decongestants. No antibiotics were permitted during study.

OUTCOMES AND MEASUREMENTS

Baseline measurements

To characterise the upper and lower airway disease and identify potential predictive factors that could influence therapeutic effectiveness, the following measurements were made at baseline. Presence and severity of asthma was made by history, spirometry (MicroMedical SuperSpiro, Chatham, Maritime, Kent, United Kingdom)(267), body plethysmography (Jaeger MasterScreen, CareFusion, Basingstoke, Hampshire, United Kingdom)(268), tidal and nasal nitric oxide (Niox®, Aerocrine, Solna, Sweden)(223), and bronchial methacholine

challenge(269). Aspirin sensitivity was diagnosed by history and nasal lysine-aspirin challenge(102). Rhinosinusitis extent was staged by computerised tomography scans of the paranasal sinuses. Computed Tomography (CT) scans of the paranasal sinuses were scored using the modified Lund-Mackay system based on reconstructed axial sections(270). Atopy was evaluated by total serum immunoglobulin E (IgE), RAST for specific IgE to grass pollen, house dust mite, cat, dog and aspergillus and immunoglobulin G (IgG) to aspergillus. A polyp biopsy to determine tissue eosinophil count was performed. Specific IgE to serum *staphylococcus aureus* enterotoxins A, B and TSST-1 (inter-assay CV 4.3%) levels were also measured (UniCAP, Phadia,Uppsala, Sweden).

Longitudinal measurements

Primary and secondary efficacy and safety outcomes were measured at randomisation (baseline) and after each treatment period i.e. at 2 weeks, 10 weeks and 28 weeks from baseline.

The primary outcome measure was nasoendoscopic polyp grading between prednisolone and placebo groups, relative to baseline. Nasoendoscopy was performed using a 2.7 mm 30° endoscope (Karl Storz-Endoskope; Tuttlingen, Germany) with an integrated endoscopy camera system (LCH 01-D, Xion Medical, Berlin, Germany) and standard video sequences were stored on a computer. These were viewed by two independent observers blinded to subject, treatment and sequence. Disagreements were resolved by discussion. Inter-rater reliability using a weighted Kappa score was 0.75 (SEM= 0.079). Secondary efficacy outcomes were: a 100mm visual analogue scale for hyposmia, Pocket Smell Test® (Sensonics, Inc. New Jersey, United States)(242), total nasal symptoms score(271), peak nasal inspiratory flow rate(13), Juniper mini rhinoconjunctivitis quality of life

questionnaire (231), Serum Eosinophil Derived Neurotoxin (intra-assay coefficient of variation 9.1%, inter-assay coefficient of variation 20%) and serum high-sensitivity C-reactive Protein (HS-CRP; intra-assay coefficient of variation 8.5, inter-assay coefficient of variation 16%) levels were measured using commercially available immunoassays (Enzyme-linked immunosorbent assay, Immunodiagnostik AG, Bensheim, Germany; Enzyme-linked immunosorbent assay, Kalon Biological Ltd, Guildford, United Kingdom respectively).

Secondary safety measures included overnight (10:00 PM to 8:00 AM) urinary free cortisol, overnight urinary cortisol corrected for creatinine (intra-assay and inter-assay coefficient of variation 9% and 6.2% for cortisol, and 2.9% and 4.6% for creatinine with no cross sensitivity with fluticasone), 8:00 AM serum cortisol, low dose 1µg adrenocorticotrophic hormone stimulation test and markers of bone turnover: serum Osteocalcin (interassay coefficient of variation 10%; immunoradiometric assay, Diasorin, Bracknell, United Kingdom), Procollagen-1 N-Terminal Peptide (P1NP; intra-assay coefficient of variation 5.8%, inter-assay coefficient of variation 6.4%, radioimmunoassay, Orion Diagnostica Oy, Espoo, Finland) and Procollagen-3 N-Terminal Peptide (P3NP; intra-assay coefficient of variation 3%, inter-assay coefficient of variation 4.6%, radioimmunoassay, Orion Diagnostica Oy, Espoo, Finland).

STATISTICAL ANALYSIS

Analyses were performed on an intent-to-treat basis and last observation carried forward principle. The study was powered at > 90 % with an alpha-error of 0.05 (two-tailed) in order to detect a 0.5 unit difference in the endoscopy polyp grading score between randomized treatments at two weeks, with an estimated sample size of 30 participants in each group using a parallel design and assuming the SD to be 0.4

units(272). This also provided a > 90% power to detect a 6 mm improvement (minimal important difference) in the hyposmia visual analogue scale score. Each outcome was assessed for normality using the Shapiro-Wilk test and by visual inspection of histograms and Q-Q plots, with consideration of previous datasets and literature. Non-normal data were logarithmically transformed where appropriate except for the hyposmia VAS where a square root transformation was employed. An analysis of covariance (ANCOVA) was performed at each time point with subject and treatment as factors and adjusted for baseline value. Polyp grading was assessed non-parametrically. Minimal important differences (MID) were estimated for polyp grading as 1 unit and hyposmia VAS as 6 mm based on consensus and Cohen's small change respectively(273). Participants improving by more than 1 MID in polyp grading or their hyposmia VAS at the end of 6 months were classed as 'responders'. Minimal important difference's for mini rhinoconjunctivitis quality of life questionnaire (0.7 units), PNIF (6 L.min-1) and TNSS (0.55 units) have been described in the literature previously, but were not used to estimate response(230, 231). Responders and non-responders were evaluated using unpaired t-tests for all interval variables and Pearson's χ^2 tests for categorical data for the following outcomes: Oral steroid induction, age, duration of rhinitis, previous sinus surgery, historical and challenge based aspirin intolerance, serum IgE, systemic and tissue eosinophilia, presence of asthma, spirometry, body plethysmography, bronchial methacholine challenge, nasal and tidal nitric oxide and paranasal sinus CT scan scores.

A secondary analysis was conducted using longitudinal random effects models. This was performed to ensure non-overestimation of treatment effects at 10 and 28 weeks. In the secondary analysis, for estimating overall treatment effects between groups,

repeated measures outcomes were analysed using random effects models. This has the benefit of allowing for the correlation of measurements over time and allowing some measurements to be missing on occasions, assuming these are missing at random (MAR). The effect of time was modeled with polynomials and the best fit obtained using Akaike's Information Criterion (AIC). AIC was also employed to assess random intercepts and random slopes in all models. All models were adjusted for age and sex. The outcomes were assessed for deviations from normal distributions and suitable transformations applied. Model-based predicted means were calculated by fitting treatment by time interactions and all random effects models were implemented in PROC MIXED in SAS (version 9.1, SAS Institute Inc., Cary, North Carolina, United States) and SPSS (version 15, SPSS® an IBM® company, Chicago, Illinois, United States). In the random effects models, time in terms of weeks was found to fit a cubic best (smallest AIC), along with random intercepts and random coefficients. Most outcomes including the primary outcome of polyp grading were reasonably normally distributed. Natural log transformations were applied to OUCC, TNSS, serum HS-CRP and serum P1NP. Finally, the best transformation for Mini RQLQ was the square root transformation and for serum P3NP was the reciprocal. The number of missing measurements varied by outcome with 7% missing for the main outcome which is small and unlikely to introduce major biases. To further interrogate each time-point and to calculate between group differences, an analysis of covariance was performed at each time point with subject and treatment as factors and baseline values as covariates.

The results of the secondary analysis are not presented in this thesis, solely for the fact that the a priori analysis of choice, which this study was powered on, was the

ANCOVA analysis. The results of this analysis can be referred to in the published article.

Results

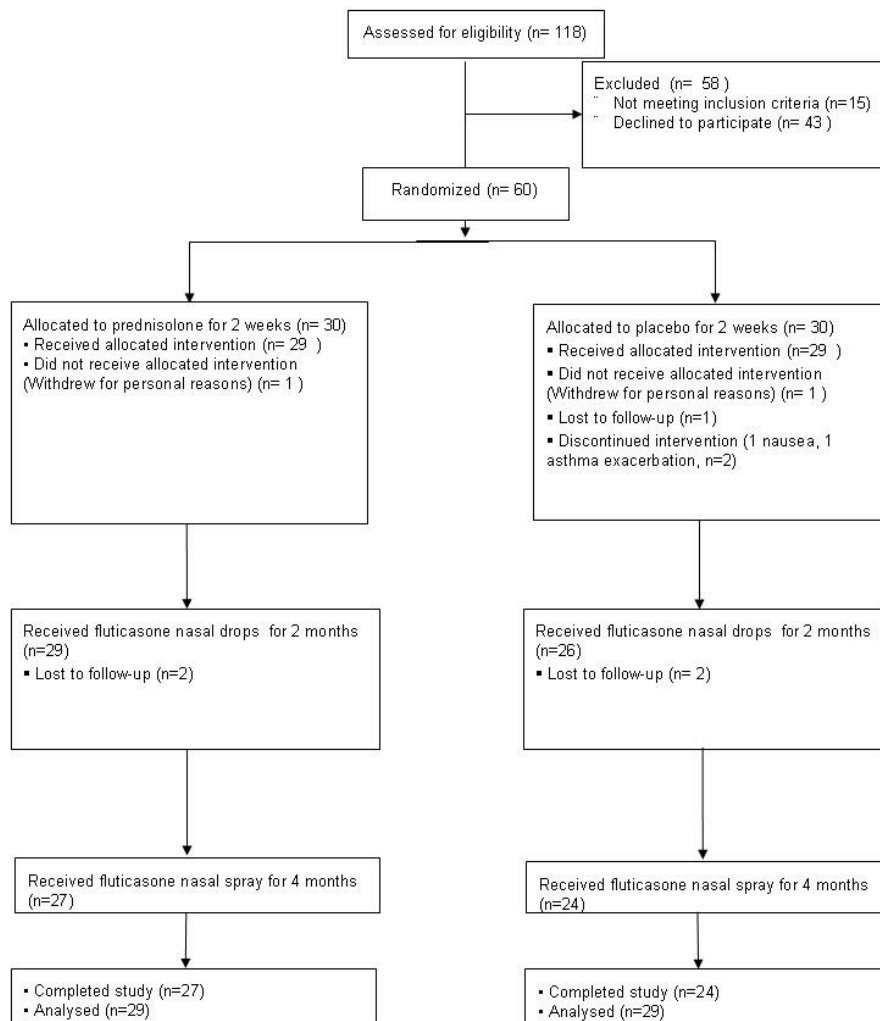


Figure 8: Participant enrolment and outcomes.

PARTICIPANTS

Of the 118 patients screened, 60 underwent randomisation and 51 completed the study (Figure 9). The study was conducted from January 2005 to February 2008. 3 patients in the prednisolone group and 4 in the placebo group had received previous oral steroids and the mean duration prior to recruitment (range) was 14 months (6-24) and 12 months (8-18) respectively. Similarly, 9 patients in the prednisolone and 10 in the placebo group had oral antibiotics in the past; mean duration prior to recruitment (range) was 17 months (2-30) and 14 months (3-25) respectively. Baseline characteristics including demographics, disease duration, upper and lower airway inflammation, airway calibre, and indices of severity such as aspirin sensitivity, atopy and asthma were similar in both treatment groups (Table 3).

Table 4: Demographics and baseline characteristics.

Variable	Prednisolone Arm N = 30	Placebo Arm N = 30
Age — yr		
Mean	49	52
Range	24—70	17—78
Male — no. (%)	14 (47)	20 (67)
Rhinosinusitis Duration — yr	11 (11)	17 (15)
Previous Surgery — no. (%)	7 (23)	9 (30)
Previous oral steroids — no. (%)	3 (10)	4 (13)
Previous oral antibiotics — no. (%)	9 (30)	10 (33)
Historical Aspirin Intolerance — no. (%)	7 (23)	6 (20)
Nasal Lysine-Aspirin Challenge Positive — no. (%)	16 (53)	15 (50)
Atopic — no. (%)	13 (43)	16 (53)
House Dust Mite	12 (40)	12 (50)
Grass Pollen	5 (17)	6 (20)
Cat	9 (30)	8 (26)
Dog	9 (30)	7 (23)
Aspergillus	2 (6)	3 (10)
Serum IgE — kU.L ⁻¹ †	93.32 (58.88—147.91)	101.71 (56.23—177.83)
Blood Eosinophil Count — cells x 10 ⁹ .L ⁻¹ †	0.34 (0.27—0.42)	0.35 (0.28—0.43)
Tissue Eosinophil Count — cells.4HPF ⁻¹ †	70.79 (35.48—141.25)	41.16 (20.63—82.13)
Aspergillus IgG — kU.L ⁻¹ †	9.60 (4.70—19.60)	12.81 (9.71—16.88)
Asthmatics — no. (%)	11 (37)	16 (53)
BDP dose equivalent—µg‡	400 (400—800)	700 (400—1250)
Spirometry		
FEV ₁ — % of predicted value	95.6 (15.8)	93.2 (17.2)
FEF ₂₅₋₇₅ — % of predicted value	78.7 (26.3)	75.6 (29.6)
FEV ₁ /FVC — %	74 (9)	83 (9)
Specific Airways Resistance — % of predicted value	97.4 (52.7)	103.3 (67.2)
AHR — no. (%)	9 (30)	11 (37)
Methacholine PC ₂₀ — mg.L ⁻¹ †	1.20 (0.56—2.58)	1.60 (0.96—1.75)
Exhaled NO — ppb†	28.32 (20.14—39.45)	32.35 (23.66—44.26)
Nasal NO — ppb†	426.58 (338.84—524.81)	392.28 (295.12—501.19)
CT Scan score	25.1 (10.8)	22.5 (10.6)
Serum IgE to <i>S. Aureus</i> enterotoxin A — kUA.L ⁻¹ †	0.04 (0.02—0.07)	0.06 (0.03—0.10)

Serum IgE to <i>S. Aureus</i> enterotoxin B — kU.A.L ⁻¹ †	0.05 (0.02—0.14)	0.05 (0.03—0.10)
Serum IgE to <i>S. Aureus</i> enterotoxin TSST — kU.A.L ⁻¹ †	0.26 (0.14—0.46)	0.24 (0.13—0.43)

* Values are arithmetic means (SD) except as indicated.

Abbreviations: IgE— immunoglobulin E; IgG — immunoglobulin G; BDP — chlorofluorocarbon beclomethasone dipropionate equivalent units; HPF— high power field; FEV₁ — forced expiratory volume in 1 second; FEF₂₅₋₇₅ — forced expiratory flow 25 -75 %; FVC — forced vital capacity; AHR— airway hyperresponsiveness defined by a positive methacholine bronchial challenge; PC₂₀— provocative concentration of methacholine causing a 20% drop in post-diluent baseline FEV₁; NO — nitric oxide; CT — computed tomography. Atopy refers to positive serum RAST testing for total IgE (>100 kU.L⁻¹) or a positive specific IgE (> 0.35 kU.L⁻¹).

† Geometric mean (95% Confidence Intervals).

‡ Dose of inhaled corticosteroid expressed as median (IQR) of chlorofluorocarbon beclomethasone dipropionate equivalent units.

Table 5: Efficacy outcomes at baseline and after each treatment period.

Variable	Prednisolone Arm N = 30	Placebo Arm N = 30	P value
Endoscopy Score (0-6)			
Baseline	4.7 (4.3—5.0)	4.8 (4.4—5.2)	0.66
Post tablets	2.6 (2.1—3.1)§	4.7 (4.4—5.0)	<0.001
Post nasal drops	2.2 (1.6—2.8)‡	3.2 (2.8—3.5)†	0.002
Post nasal spray	2.8 (2.2—3.4)†	3.3 (2.8—3.7)†	0.04
Hyposmia VAS (0-100 mm)			
Baseline	58.64 (46.48—72.22)	53.14 (39.27—69.03)	0.59
Post tablets	27.52 (18.37—38.53)§	54.55 (41.29—69.64)	<0.001
Post nasal drops	27.24 (17.41—39.25)‡	38.12 (26.31—52.10)†	0.05
Post nasal spray	29.27 (19.84—40.53)‡	41.33 (28.96—55.92)	0.02
Pocket Smell Test® Score (0-3)			
Baseline	1.65 (1.10—2.21)	1.54 (1.01—2.07)	0.53
Post tablets	2.50 (2.13—2.86)‡	1.58 (1.03—2.13)	0.04
Post nasal drops	2.50 (2.13—2.86)‡	2.04 (1.53—2.55)†	0.40
Post nasal spray	2.31 (1.84—2.78)†	1.67 (1.12—2.21)	0.34
TNSS (units)			
Baseline	3.39 (2.35—4.43)	3.26 (2.19—4.33)	0.43
Post tablets	1.03 (0.40—1.67)§	3.22 (1.86—4.57)	<0.001
Post nasal drops	1.14 (0.69—1.59)§	1.30 (0.69—1.91)‡	0.008
Post nasal spray	1.00 (0.58—1.41)§	1.54 (0.95—2.14)†	0.14
Mini RQLQ (units)			
Baseline	1.62 (1.23—2.01)	2.17 (1.51—2.83)	0.75
Post tablets	0.75 (0.33—1.16)§	1.73 (1.21—2.24)	0.001
Post nasal drops	0.75 (0.46—1.03)§	0.69 (0.45—0.94)§	0.25
Post nasal spray	0.66 (0.45—0.87)§	1.08 (0.72—1.44)‡	0.12
PNIF (L.min ⁻¹)			
Baseline	107.08 (92.96—121.20)	122.35 (85.39—159.32)	0.22
Post tablets	146.67 (129.43—163.90)§	132.94 (100.08—165.80)	0.003
Post nasal drops	148.33 (131.77—164.93)§	160.29 (125.91—194.67)‡	0.64
Post nasal spray	146.88 (129.00—164.75)§	146.47 (110.34—182.59)	0.27
Serum EDN (ng.L ⁻¹)¶			
Baseline	32.22 (25.28—38.31)	31.21 (23.26—40.50)	0.99
Post tablets	25.28 (19.43—32.89)‡	32.90 (25.63—42.52)	0.01
Post nasal drops	30.48 (24.25—38.05)	29.86 (24.08—37.27)	0.91
Post nasal spray	31.12 (18.37—37.01)	38.32 (30.48—48.50)	0.25
Serum HS-CRP (mg.L ⁻¹)¶			
Baseline	0.77 (0.53—1.11)	1.13 (0.75—1.70)	0.10
Post tablets	0.59 (0.46—0.78)	1.00 (0.65—1.51)	0.005
Post nasal drops	0.96 (0.66—1.40)	1.20 (0.82—1.76)	0.16
Post nasal spray	0.96 (0.68—1.36)	1.12 (0.77—1.64)	0.32

Serum IgE to <i>S. Aureus</i> enterotoxin			
A — kUA.L ⁻¹ ¶			
Baseline	0.04 (0.02—0.07)	0.06 (0.03—0.10)	0.95
Post tablets	0.04 (0.02—0.07)	0.06 (0.03—0.10)	0.93
Serum IgE to <i>S. Aureus</i> enterotoxin			
B — kUA.L ⁻¹ ¶			
Baseline	0.05 (0.02—0.14)	0.05 (0.03—0.10)	0.39
Post tablets	0.05 (0.02—0.13)	0.06 (0.04—0.11)	0.86
Serum IgE to <i>S. Aureus</i> enterotoxin			
TSST — kUA.L ⁻¹ ¶			
Baseline	0.26 (0.14—0.46)	0.24 (0.13—0.43)	>0.99
Post tablets	0.25 (0.13—0.45)	0.26 (0.15—0.44)	0.86

* Data shown are arithmetic means (95 % Confidence Intervals) except where indicated. P values are for

between-group comparisons for each outcome measure at sequential timepoints.

† P < 0.05 for within-participant comparisons from respective baselines.

‡ P < 0.01 for within-participant comparisons from respective baselines.

§ P < 0.001 for within-participant comparisons from respective baselines.

¶ Geometric mean (95% Confidence Interval).

Abbreviations: VAS— visual analogue scale; TNSS— total nasal symptom score; RQLQ— Juniper mini-rhinoconjunctivitis quality of life questionnaire; EDN— eosinophil derived neurotoxin; HS-CRP— high sensitivity C-reactive protein; *S. Aureus*— Staphylococcus Aureus.

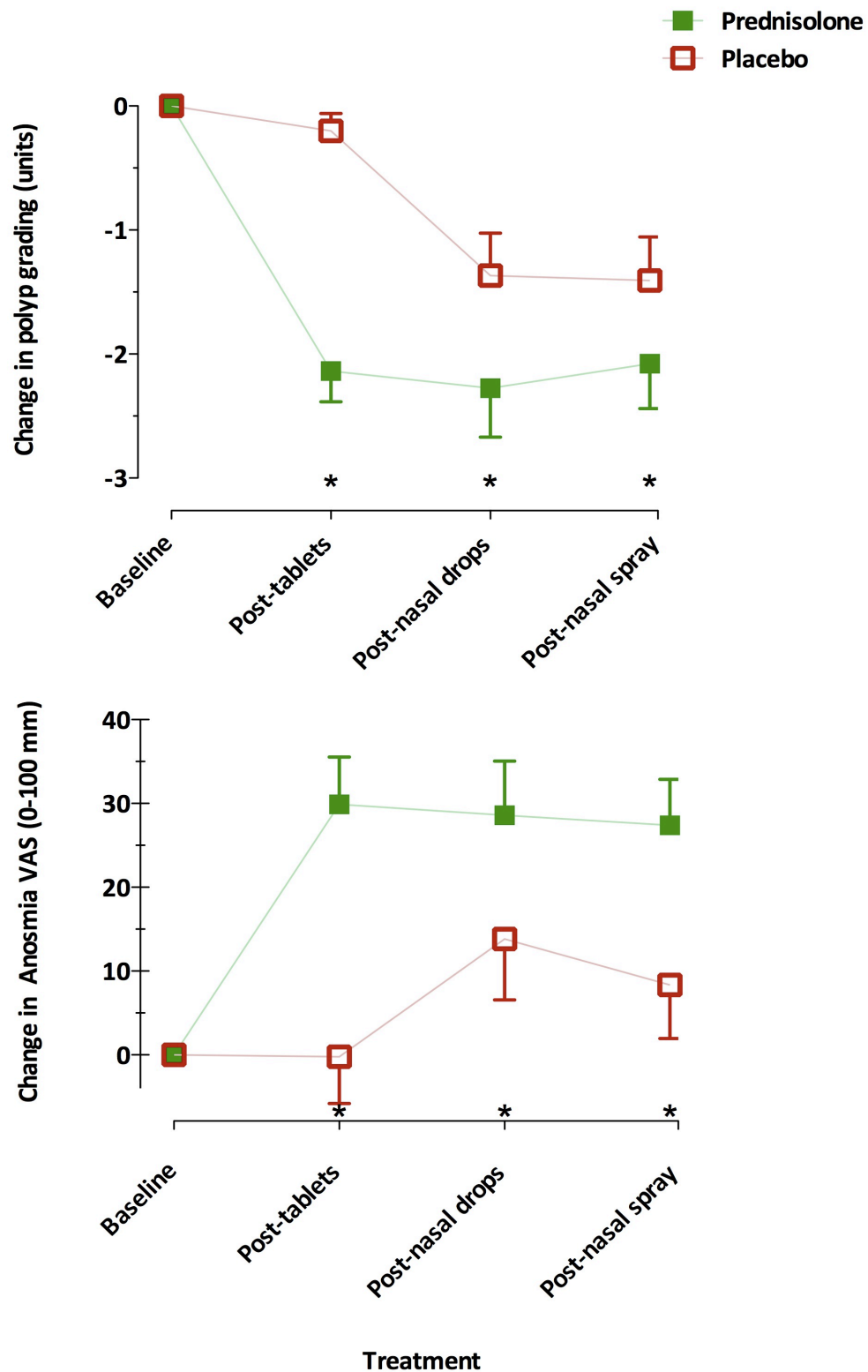


Figure 9: Efficacy outcomes.

Mean values of efficacy outcomes after each stage of treatment.

* denotes an overall significant mean difference between groups.

Values at each timepoint and changes in primary and secondary efficacy outcomes are presented in Table 4 and Figure 6. The percentage of missing data for polyp grading for the prednisolone group was: 3% at 2 weeks, 7% at 10 weeks, 4% at 28 weeks, and for the placebo group was: 7% at 2 weeks, 7% at 10 weeks and 4% at 28 weeks. The mean decrease in polyp grade from baseline to 2 weeks was 2.1 units (SD 1.1) for the prednisone group and 0.1 units (SD 1.0) for the placebo group (mean difference between groups, -1.8 units (95% CI -2.4 , -1.2 ; $P < 0.001$)). The difference between groups at 10 and 28 weeks was: -1.08 units (95% CI -1.74 , -0.42 ; $P = 0.001$) and -0.8 units (95% CI -1.8 to 0.2 ; 0.11). Mean decrease in hyposmia score from baseline to 2 weeks was 31.12 mm (SD 30.1) for the prednisolone group and 1.41 mm (SD 30.6) for the placebo group (mean difference between groups, -28.33 mm (95% CI -42.71 , -13.96 ; $P = 0.002$)). The difference between groups at 10 and 28 weeks was: -16.06 mm (95% CI -30.99 , -1.13 ; $P = 0.03$) and -12.13 mm (95% CI -30.55 , 6.29 ; $P = 0.19$).

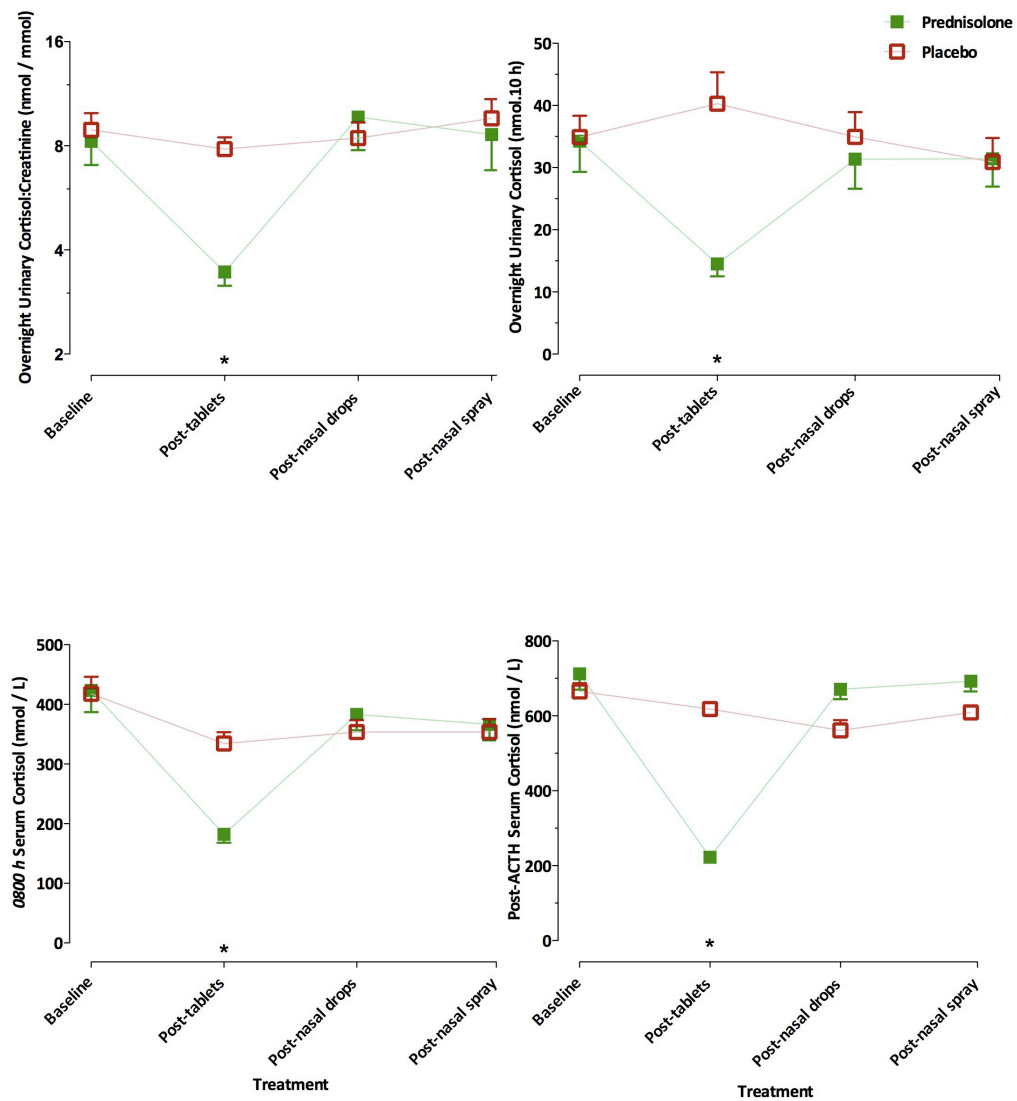


Figure 10: Safety outcomes.

Mean values of safety outcomes after each stage of treatment.

* denotes an overall significant mean difference between groups.

SAFETY OUTCOMES

Table 6: Adverse events.

Event	Prednisolone arm	Placebo arm
Epistaxis triggered by nasal spray	2	1
Viral rhinitis	6	6
Facial pain	1	1
Tonsillitis	0	1
Headache	2	1
Flu-like symptoms	2	5
Rash	1	1
Conjunctivitis	1	0
Musculoskeletal pain	4	4
Nausea	0	1
Asthma exacerbation	0	1

37 participants (19 in prednisolone and 18 in the placebo group) reported an adverse event.

No serious adverse events (SAE) were reported. An SAE was defined as one of the following: an event causing the death of the participant, a life-threatening event, hospitalization, persistent or significant disability affecting important life functions, congenital anomaly or birth defect in the offspring of a woman treated before or during pregnancy, pregnancy, and important medical events requiring urgent and intensive intervention to prevent one of the outcomes listed in the definition above.

Following randomization, 1 participant withdrew because of nausea and another had an asthma exacerbation ('other medical reasons' in Figure 9). 37 participants (19 in prednisolone and 18 in the placebo group) reported an adverse event. Adverse events did not differ between groups (Table 5). There were no serious adverse events as defined in the protocol. Basal and dynamic adrenal function was suppressed by oral prednisolone but recovered after switching to nasal drops (Table 6 and Figure 11). Overnight urinary cortisol corrected for creatinine (OUCC) was suppressed to 50% of its baseline value and ACTH stimulated serum cortisol suppressed by 86%

following 2 weeks of oral prednisolone treatment. At 10 and 28 weeks, however, there was no significant residual adrenal suppression compared to baseline. Markers of osteoblastic activity showed a similar transient decrement during oral steroid therapy, with a return to baseline with subsequent topical treatment (Table 6).

Table 7: Safety outcomes at baseline and after each treatment period.

Variable	Prednisolone Arm N = 30	Placebo Arm N = 30	P
OUCC (nmol.mmol ⁻¹)§			
Baseline	8.07 (6.27—10.40)	8.70 (7.04—10.73)	0.93
Post tablets	3.14 (2.47—3.97)‡	7.62 (6.39—9.09)	<0.001
Post nasal drops	7.74 (5.79—10.37)	7.48 (5.98—9.35)	0.68
Post nasal spray	8.51 (6.36—11.35)	8.14 (6.38—10.42)	0.83
OUC (nmol.10 h)			
Baseline	31.74 (22.78—40.69)	36.23 (28.13—44.34)	0.93
Post tablets	15.26 (10.58—19.93)†	38.94 (28.56—49.33)	0.009
Post nasal drops	27.62 (19.75—35.49)	35.51 (26.10—44.03)	0.89
Post nasal spray	29.54 (21.60—37.48)	30.90 (22.87—38.93)	0.14
Pre-ACTH 0800 h Serum Cortisol (nmol.L ⁻¹)			
Baseline	435.03 (352.34—517.73)	391.25 (334.66—447.84)	0.64
Post tablets	184.76 (152.61—216.91)‡	327.81 (284.53—371.08)	<0.001
Post nasal drops	387.01 (329.13—444.89)	345.46 (303.60—387.31)	0.72
Post nasal spray	370.96 (313.86—428.06)	353.57 (308.38—398.75)	0.65
Post-ACTH Serum Cortisol (nmol.L ⁻¹)			
Baseline	736.56 (645.85—827.27)	661.77 (608.67—714.87)	0.32
Post tablets	225.83 (191.96—259.70)‡	616.23 (584.65—647.81)	<0.001
Post nasal drops	670.01 (606.53—733.49)	590.47 (544.34—636.60)	0.10
Post nasal spray	684.98 (627.87—742.10)	611.00 (570.71—651.30)	0.18
Serum Cortisol increment (nmol.L ⁻¹)			
Baseline	289.51 (209.32—369.71)	246.26 (193.24—299.28)	0.56
Post tablets	39.22 (26.39—52.04)‡	273.34 (231.57—315.11)	<0.001
Post nasal drops	294.88 (249.82—339.94)	207.32 (146.28—268.36)	0.25
Post nasal spray	309.30 (258.63—359.96)	219.06 (157.80—280.32)	0.24
Serum Osteocalcin (nmol.L ⁻¹)			
Baseline	1.06 (0.93—1.18)	1.23 (1.04—1.42)	0.20
Post tablets	0.72 (0.55—0.88)‡	1.15 (0.97—1.32)	0.03
Post nasal drops	1.03 (0.89—1.18)	1.33 (1.14—1.52)	0.29
Post nasal spray	1.08 (0.91—1.24)	1.39 (1.11—1.68)	0.27
Serum P1NP (µg.L ⁻¹)			
Baseline	31.13 (27.55—34.71)	36.78 (31.85—41.71)	0.19
Post tablets	23.40 (20.70—26.09)	38.31 (33.13—43.48)	0.003
Post nasal drops	28.40 (24.96—31.85)	39.45 (33.23—45.67)	0.12

Post nasal spray	30.81 (27.02—34.59)	41.39 (31.92—50.85)	0.16
Serum P3NP ($\mu\text{g.L}^{-1}$)			
Baseline	2.38 (2.19—2.56)	2.67 (2.38—2.96)	0.88
Post tablets	2.18 (1.96—2.40)‡	2.78 (2.38—3.18)	0.01
Post nasal drops	2.42 (2.23—2.61)	2.89 (2.31—3.47)	0.25
Post nasal spray	2.50 (2.27—2.74)	3.03 (2.47—3.59)	0.24

* Data shown are arithmetic means (95 % Confidence Intervals) unless indicated otherwise. P values are for between-group comparisons for each outcome measure at sequential timepoints.

† P < 0.05 for within-participant comparisons from respective baselines.

‡ P < 0.01 for within-participant comparisons from respective baselines.

§ Geometric means (95 % Confidence Intervals).

¶ P < 0.001 for within-participant comparisons from respective baselines.

Abbreviations: OUCC— overnight urinary cortisol creatinine ration; OUC— overnight urinary free cortisol; ACTH— synthetic adrenocorticotrophic hormone (Synacthen); P1NP— Procollagen-1 N-Terminal Peptide; P3NP— Procollagen-3 N-Terminal Peptide.

STAPHYLOCOCCUS AUREUS ENTEROTOXINS

Circulating IgE antibodies to serum *S.aureus* enterotoxins A (SEA), B (SEB) and TSST-1 levels were detected in 53%, 53% and 68% of participants respectively. SEA and TSST-1 were not statistically different between groups as 0.11 kUA.L^{-1} (–0.01, 0.23) and 0.52 kUA.L^{-1} (–1.15, 0.31) respectively. Levels of SEB were significantly higher in the subgroup of participants with a positive history of aspirin intolerance as mean difference (95%CI) 0.16 kUA.L^{-1} (0.01, 0.32). Enterotoxin IgE levels were not different between nasal lysine-aspirin challenge positive and negative groups. Levels of enterotoxin-specific IgE did not alter with systemic corticosteroid therapy (Table 4, Figure 12).

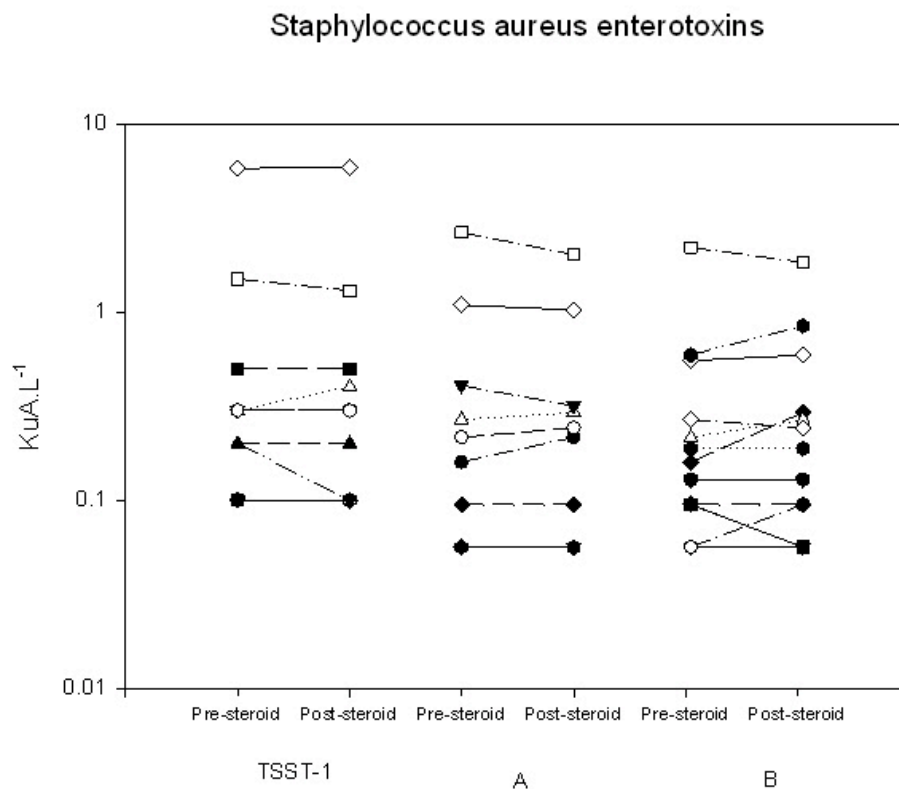


Figure 11: S aureus enterotoxin specific IgE pre and post oral steroids.

Levels of *staphylococcus aureus* enterotoxin-specific IgE did not change ($P > 0.05$) with oral prednisolone. The figure above shows individual patients within the prednisolone arm only before and after receiving 25 mg of oral prednisolone once daily for 2 weeks. Symbols are individual patient arithmetic means. No significant mean difference was demonstrated.

There were no differences in baseline predictors between responders and non-responders at 6 months. (Table 7). Twenty five (83%) of participants in the prednisolone group responded to therapy as indicated by an improvement by more than 1 MID in either polyp grade or hyposmia visual analogue scale at the end of 28 weeks (“responders”) as compared to 17 (57%) of participants in the placebo group.

Table 8: Comparisons between responders and nonresponders at 28 weeks.

Variable	Responders N = 42	Non-responders N = 18	P value
Steroid induction group — no. (%)	25 (60)	5 (17)	0.03
Age — yr	51(13)	49(13)	0.55
Previous Surgery — no. (%)	9 (22)	7 (39)	0.16
Rhinosinusitis Duration — yr	14(13)	17(14)	0.53
Historical Aspirin Intolerance — no. (%)	10 (24)	3 (17)	0.54
Nasal Lysine-Aspirin Challenge Positive — no. (%)	20 (48)	11 (61)	0.34
Atopic — no. (%)	13 (43)	16 (53)	0.44
Serum IgE — kU.L ⁻¹ †	109.17(87.36 — 136.5)	70.79(54.45 — 92.02)	0.31
Blood Eosinophil Count — cells x 10 ⁹ .L ⁻¹ †	0.33(0.30 — 0.36)	0.40(0.37 — 0.43)	0.25
Tissue Eosinophil Count — cells.4HPF ⁻¹ †	64 (41 — 102)	28 (7 — 117)	0.31
Aspergillus IgG — kU.L ⁻¹ †	11.35(9.08 — 14.18)	9.88(6.29 — 15.51)	0.78
Asthmatics — no. (%)	19 (45)	8 (44)	0.95
Spirometry			
FEV ₁ — % of predicted value	92.9(16.9)	98.8(14.7)	0.19
FEF ₂₅₋₇₅ — % of predicted value	73.7(27.3)	85.3(27.3)	0.14
FEV ₁ /FVC — %	74(6.5)	76(8.4)	0.61
Specific Airways Resistance — % of predicted value	106.1(63.0)	88.6(60.9)	
AHR — no. (%)	15 (36)	5 (29)	0.64
Methacholine PC ₂₀ — mg.L ⁻¹ †	1.28(1.04 — 1.57)	1.82(1.07 — 3.09)	0.55
Baseline CT Scan score	23 (2)	22 (3.5)	0.72

* Values are arithmetic means (SD) except as indicated. Numbers (%) indicate number (%) treated in either prednisolone or placebo groups.

Responders were participants in both groups and were defined as having more than 1 ‘minimal important difference’ improvement in their polyp score (1 unit) or anosmia visual analogue score (6 mm).

P values represent comparisons between responders and nonresponders using unpaired Student t-tests for all interval variables and Pearson’s χ^2 tests for categorical data.

† Geometric mean (95% Confidence Intervals).

Abbreviations: IgE, immunoglobulin E; HPF, high power field; FEV₁ — forced expiratory volume in 1 second; FEF₂₅₋₇₅ — forced expiratory flow 25 -75 %; FVC — forced vital capacity; AHR— airway hyperresponsiveness defined by a positive methacholine bronchial challenge; PC₂₀— provocative concentration of methacholine causing a 20% drop in post-diluent baseline FEV₁; CT — computed tomography.

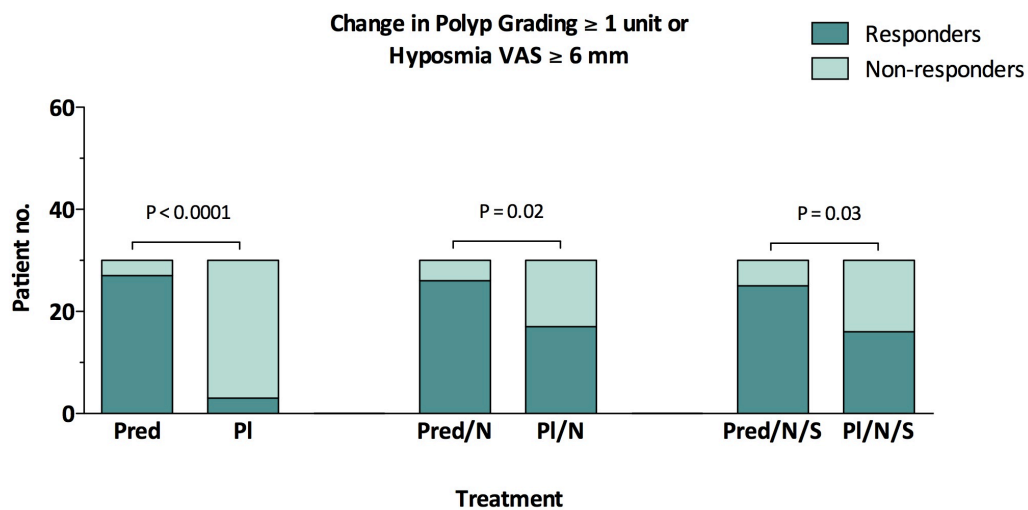


Figure 12: Proportional change in responders and nonresponders at each stage of treatment.

Change in polyp grade and hyposmia VAS score from respective baseline values in the prednisolone (Pred) and placebo (PI) arms at each stage of treatment. Responders were defined as a change in polyp grading > 1 unit or an improvement in the Hyposmia VAS score of more than or equal to 6 mm. The graph demonstrates a significantly higher proportion of responders among participants who had received initial oral steroids as compared to placebo at each stage of treatment at 2, 10 and 28 weeks i.e. after oral steroids (Pred or PI), after nasal steroid drops (N) and nasal steroid spray (S), respectively.

Discussion

At 6 months, patients with CRSwNP who underwent oral prednisolone induction showed a sustained and significantly greater improvement in nasal function, systemic inflammation and quality of life parameters, without compromising systemic safety than those with sham-induction. These benefits were not modified by indices of disease severity such as aspirin intolerance, atopy, duration of illness, and concomitant asthma.

Our study demonstrates a parallel and sustained improvement in polyp size and the sense of smell. This unique finding was supported objectively using the Pocket Smell Test® which showed a similar trend within the prednisolone group, although it did not reach statistical significance between groups. This is particularly important, as nasal obstruction and hyposmia constitute the two primary symptoms of CRSwNP and have the most significant impact on the patient's quality of life(1, 239).

CRSwNP causes olfactory impairment due to mechanical obstruction, sensorineural defects secondary to mucosal inflammation and stem cell loss(239). When treated with topical steroids alone, improvement in the patient's sense of smell often 'lags behind' the improvement in nasal obstruction(240), possibly due to limited access to the olfactory cleft. This disconnect between congestion and olfaction is even more apparent following endoscopic sinus surgery(241). Indeed, after surgical treatment, patients' sense of smell has been known to worsen or in the rare instance disappear altogether(274). Systemic steroid induction overcomes the deficiencies of both these approaches. Indeed, there is evidence that oral steroids have a direct stimulatory effect on olfactory neurons(275). However, the sustained improvement observed in

this study with sequential topical corticosteroids points to a reduction in local mucosal inflammation and oedema as a more likely mechanism.

A literature search of MEDLINE (1950 to present), EMBASE (1996 to present) and COCHRANE CENTRAL did not yield any randomized controlled clinical trials in the long-term treatment of CRSwNP. *Hissaria et. al.* conducted a randomized controlled trial evaluating the efficacy of 2 weeks of oral prednisolone in nasal polyposis(256). In an uncontrolled trial by *Benitez et. al.* 63 participants receiving 2 weeks of oral prednisolone followed by 12 weeks of topical steroid showed overall symptomatic improvement, while the 21 participants in the control group received no treatment(276). Neither examined the importance of systemic induction on long term maintenance topical corticosteroid therapy with a comprehensive array of efficacy and safety outcomes.

We chose a dose of 25mg/day of prednisolone for induction as it provides good systemic anti-inflammatory effect and is available as a single, once daily tablet. This regimen is frequently used in our clinical practice to aid compliance and reduce potential side-effects of higher doses, such as sleep disturbance. Indeed, in our study no adverse events attributable to oral steroids were reported. We hypothesized that improvement in CRS with NP would require adequate clearance of the ostiomeatal complex, and therefore steroid induction was followed by 2 months treatment with intranasal drops. Compared to nasal sprays, drops provide better deposition to the ostiomeatal complex with a low systemic bioavailability(154, 277). However, they are relatively expensive and not universally available. For this reason participants were maintained on intranasal spray for the remainder of the study.

Whilst we demonstrated additional long-term efficacy with initial systemic induction, it is perhaps of greater importance to the clinician to know if this is a safe approach. Indeed, more than 50% of our patients had concomitant asthma and were on inhaled corticosteroids, similar to figures reported in literature(13). To our knowledge, there are no long-term studies evaluating the hypothalamic-pituitary-adrenal axis or bone turnover in CRSwNP patients from the general population. Reassuringly, we found no residual adrenal suppression or reduction in osteoblastic activity with our treatment regimen at 2 and 6 months. Whereas previous studies in healthy volunteers with fluticasone nasal drops and spray have shown low systemic bioavailability even at supranormal dosages, this cannot be generalised to patients with CRSwNP(154). The pan-mucosal inflammation and impaired mucociliary clearance characteristic of CRSwNP may influence nasal retention, corticosteroid absorption and systemic bioavailability, and the use of concomitant inhaled corticosteroid may add to the systemic steroid burden.

Crucially, this paper brings into focus some of the wider issues around current international guidelines for the management of CRSwNP. For instance, current guidelines do not advocate the use of oral steroid induction as an initial approach to treatment(13). Indeed, in accordance with these guidelines, a patient who presents to a primary care physician would be treated with monotherapy with intranasal corticosteroids and then referred on to a specialist if there is no significant improvement in symptoms. As demonstrated by our study, without the initial induction, such patients with polyposis will have been undertreated, as the crucial ostiomeatal complex obstruction would not have been addressed. This study therefore, highlights the need to establish a more unified; evidence based approach to

CRS therapy, and suggests that an initial induction and maintenance regime may be more appropriate even in mild-moderate disease for long-lasting benefit and to reduce specialist referrals and hospital costs.

Interestingly, in half of our patients we detected serum IgE to *staphylococcus aureus* enterotoxins (SAE). Ever since they were first detected in nasal polyp tissue homogenates, there has been growing fascination with these ‘superantigens’, in particular their ability to drive the persistent eosinophilic airways inflammation, characteristic of CRSwNP, through multiclonal IgE activation(50, 278). Similar findings have been reported in atopic dermatitis, asthma and COPD, suggesting a significant role of SAEs in eosinophilic inflammatory conditions and the unified airway(13). Indeed, patients with concomitant asthma or aspirin intolerance have higher detectable levels of these superantigens compared to CRS alone, corresponding to the greater burden of eosinophilic inflammation in these conditions(46). In the present study, we found significantly higher serum SAE levels in patients reporting a positive history of aspirin induced symptoms compared to those without a positive history. However, this was not seen in patients who had a positive nasal aspirin challenge. This could be due to a milder degree of aspirin sensitivity being detected by a positive nasal aspirin challenge as, it is well known that the prevalence of aspirin intolerance based on challenge testing is higher than by verbal history alone(93). Ours is the first randomized clinical trial to evaluate if systemic corticosteroid therapy influences the serum specific IgE to these superantigens. A lack of effect observed in our study suggests that either corticosteroids influence eosinophilic inflammatory pathway further downstream, or that disease modification by superantigens is principally a local process. Further, our

results also show that systemic markers of eosinophil activation (EDN) and inflammation (HS-CRP) were suppressed by systemic but not topical corticosteroid therapy. This in turn perhaps suggests that nasal drops influenced polyp reduction by a direct topical anti-inflammatory effect rather than any systemic effect on eosinophil function.

We recognise the limitations of our study. We did not use a detailed validated measure of olfaction such as the 40-item University of Pennsylvania Smell Identification Test (UPSIT), but the decision to use a combination of a subjective visual analogue score and the Pocket Smell Test ® was based on subject visit length. It is also well-recognized that the UPSIT has some scents which are not indigenous to the UK. We did not measure nasal inflammation using surrogates such as nitric oxide, eosinophils from repeated biopsies or cytokines obtained from nasal lavage. We believe that while there is a plethora of literature using these measures, their clinical relevance is still unclear. To this end, we used a clinically meaningful set of subjective, objective and quality of life efficacy outcomes. Direct assessment of the ostiomeatal complex using serial CT scanning would have been desirable, but does not reflect routine clinical practice in the UK, where the majority of nasal polyposis are treated in primary care and such investigations are not readily accessible. Moreover, this would have exposed patients to an unacceptable level of radiation, and would be unlikely to add much to endoscopy scoring. We used the Juniper rhinoconjunctivitis questionnaire as a quality of life assessment tool. This might seem unorthodox in the presence of other disease-specific questionnaires like the SNOT-20(236). However, since 50% of our patients were atopic, it was felt to be a comprehensive tool to evaluate quality of life.

Future studies should consider whether an ‘induction and maintenance’ approach should be considered at the point of first diagnosis of CRS with NP (e.g., as made in primary care) and whether it is beneficial in milder disease, where ostiomeatal complex obstruction is less severe. Short-term nasal decongestants may further improve access of intranasal corticosteroids and should be assessed in conjunction with prednisolone induction. Finally, large long-term studies are required to assess whether steroid induction can delay or reduce the need for surgical intervention and influence post-surgical recurrence rates.

To summarise, the present study shows that oral corticosteroid induction followed by intranasal maintenance therapy is more effective than topical therapy alone as recommended by current guidelines. This can be achieved without lasting adverse effects of corticosteroids. This model could serve as an initial treatment approach for nasal polyposis in primary care.

Chapter 5: To evaluate if a positive
nasal lysine-aspirin challenge
predicts a more severe phenotype of
chronic rhinosinusitis

Introduction

Chronic rhinosinusitis with nasal polyposis (CRSwNP) is one of the commonest chronic respiratory disorders with significant morbidity, major healthcare costs and a high socioeconomic burden(26). Aspirin intolerant rhinosinusitis (AIR) is a clinical syndrome characterized by a combination of CRSwNP with or without asthma; and precipitation of asthma and rhinitis attacks after ingestion of aspirin and most of the nonsteroidal anti-inflammatory drugs (NSAIDs)(100). Around 5 to 8% of patients with CRSwNP give a history of aspirin intolerance and almost invariably have associated asthma; this trio of disorders is often referred to as 'aspirin triad' or 'Samter's triad'(95, 186, 279). Aspirin intolerant patients have a greater burden of illness, worse quality of life and experience frequent disease recurrences(1, 280). Their illness is recalcitrant to medical and surgical treatment and they are often highly steroid dependant and steroid unresponsive(9, 99, 281). Establishing a diagnosis of aspirin intolerance is important as it provides the patient with a host of common drugs that must be avoided, diagnoses a particularly severe and recalcitrant form of disease phenotype, and allows a choice of specific therapy such as leukotriene modifiers or aspirin desensitisation(282). Interestingly, studies including a recent meta-analysis have shown that the prevalence of aspirin intolerance may be as high as 21% when diagnosed with aspirin provocation testing as compared to 3 - 11% with a history of intolerance(93, 102). The 2007 EAACI/GA2LEN guidelines consequently recommend greater use of aspirin provocation testing in routine clinical practice, in order to prevent under-diagnosis(102). It is, however, unclear what the indication for such testing is and to our knowledge clinicians do not routinely test for aspirin sensitivity and as such it remains a predominantly research

tool. Indeed, in a small group of nasal polyposis with (n=15) and without asthma (n=17) but without a history of aspirin intolerance, Killen and co-workers failed to show a significant prevalence of a positive bronchial aspirin challenge(283). There are no data in the literature on whether a positive aspirin challenge test is indicative of a more severe phenotype of CRSwNP in those without symptoms, making the clinical significance of conducting such a test in asymptomatic individuals dubious. We evaluated a cohort of patients with CRSwNP with and without asthma for aspirin intolerance using nasal lysine-aspirin provocation tests in accordance with current guidelines(102). We also evaluated a host of upper and lower airway parameters, quality of life and systemic bioactivity to establish factors associated with aspirin intolerance.

Methods

Participants and Settings

Non-smoking, adult patients with CRSwNP, with or without asthma attending our research group for screening were invited to participate. CRSwNP was diagnosed based on current criteria as having at least two of the following symptoms for more than 12 weeks – anterior and/or posterior nasal discharge, nasal obstruction, decreased sense of smell and presence of bilateral nasal polyposis of grade 2 or above using the Lildholdt grading(266). Asthma was defined as those with known asthma as diagnosed by primary or secondary care physicians and on treatment. Aspirin intolerance was diagnosed in those with a documented reaction or a history of ingestion of aspirin or non-steroidal anti-inflammatory drugs followed by a typical reaction. Patients who were treated with oral corticosteroid within three months, sinus surgery within one year, recent upper respiratory tract infection within 1 month, recent antibiotic course within 1 month, mechanical nasal airway obstruction due to septal deviation >50%, pregnancy and lactation were excluded. The Tayside Committee for Medical Research Ethics gave full approval and all participants gave written informed consent. No alterations were made to their regular medications.

Measurements

At screening, inclusion status was determined, medical history was taken and an ear nose and throat examination was carried out. Atopic status was established and defined as having a positive skin prick test to a panel of common aeroallergens and/or a serum IgE level of $> 100 \text{ kU.L}^{-1}$. The nasal lysine-aspirin challenge was conducted as described previously in the literature(102, 284-286). In brief, patients were challenged with 25 mg of lysine-aspirin (L-ASA, Aspisol; Bayer PLC,

Newbury, England) with direct endoscopic visualization on each inferior turbinate, in a seated position and their head tilted back. PNIF and the total nasal volume (cm^3) using an acoustic rhinometer (A1 Executive; GM Instruments Ltd, Kilwinning, Scotland), at 10-minute intervals, for 120 minutes after challenge. Prior to this, at baseline nasal symptoms, inspiratory flows and nasal volumes are recorded during the first 30 min at 10-min intervals. Then the nasal challenge with 0.9% NaCl (80 μl) instilled into each nostril under direct endoscopic visualization for assessment of nonspecific nasal hyperreactivity. Nasal symptoms, inspiratory flow and nasal volumes are measured over the following 30 min at 10- min intervals. If a change over 20% in the recorded values occurs then the upper airway was considered hyperreactive and further challenge could not take place. A positive nasal L-ASA test was defined as a 25% reduction in total nasal volume measured by acoustic rhinometry or a 40% reduction in PNIF as recommended in guidelines(102).

In order to characterise the upper and lower airway disease and to identify potential predictive factors associated with a positive challenge the following measurements were made at baseline. For the upper airway, endoscopic polyp grading using Lildholdt grading(253), Peak nasal inspiratory flow (PNIF)(205, 230, 287), total nasal symptoms score(271), Pocket Smell Test® (Sensonics, Inc. New Jersey, USA)(242), Juniper mini rhinoconjunctivitis quality of life questionnaire (Mini RQLQ)(231) and nasal nitric oxide (Niox®, Aerocrine)(223) were measured. For the lower airway , presence and severity of asthma was made by history, spirometry (MicroMedical SuperSpiro)(267), body plethysmography (Jaeger)(288), tidal nitric oxide (Niox®, Aerocrine)(223), and bronchial methacholine challenge(269).

Rhinosinusitis extent was staged by CT scans of the paranasal sinuses which were

scored using the modified Lund-Mackay system based on reconstructed axial sections(6).

For purposes of comparison, four groups were defined as CRSwNP with and without asthma with a positive or negative nasal L-ASA challenge test.

Statistical Analysis

Each outcome was assessed for normality using the Shapiro-Wilk test and by visual inspection of histograms and Q-Q plots, with consideration of previous datasets and literature. Nongaussian data were log-transformed prior to analysis to normalize the distribution. Groups were compared using an overall analysis of variance with Bonferroni correction for multiple pairwise comparisons and a two-tailed significance set at $p < 0.05$. We also conducted a multiple regression analysis using the baseline outcomes as predictors and the area under the curve of the total nasal volume curve for the 2 hours after the L-ASA challenge as the outcome variable. A multiple regression model was constructed using predictor variables shown to be significantly associated with a history of aspirin sensitivity on an initial univariate analysis or deemed of importance *a priori* from the literature or biological plausibility. The resultant model consisted of: age, sex, duration of rhinitis, polyp score, Mini RQLQ, Pocket Smell Test® score, history of aspirin intolerance and tidal NO. Residuals were tested for normality to ensure assumptions for the models had been met. Statistical analyses were performed using SPSS version 17.0 (Chicago, Illinois, USA).

Results

Baseline demographics are presented in Table 8. N = 75

Table 9: Baseline characteristics.

Variable	Value
Demographics	
Age, years – mean (range)	51 (34 – 75)
Sex, males – No. (%)	39 (52)
Duration of rhinosinusitis – years	15 (2)
Previous surgery – No. (%)	22 (29%)
Atopy – No. (%)	29 (39%)
Asthma – No. (%)	39 (52)
Methacholine responders– No. (%)	20 (27)
Baseline nasal outcomes	
CT score—units	22.8 (1.7)
Polyp score—units	4.5 (0.2)
Peak nasal inspiratory flow—L/min	114.9 (6.3)
Total nasal symptoms score—units	3.3 (0.3)
Mini RQLQ—units	1.87 (0.14)
Pocket Smell Test®—units	1.6 (0.1)
Nasal NO—ppb*	389.04 (1.07)
Baseline lower airway outcomes	
Exhaled NO—ppb*	30.47 (1.09)
FEV ₁ —% predicted	94.7 (2.1)
FEF ₂₅₋₇₅ —% predicted	75.4 (3.2)
sReff— % predicted	100.0 (7.5)
Methacholine PC ₂₀ —mg/ml	8.94 (1.22)
Serum IgE—kU/L*	97.49 (1.20)
Eosinophil count—cells/uL*	0.35 (1.07)

Data are presented as arithmetic mean (SEM) except where indicated.

* Geometric mean (SEM)

Abbreviations: CRSwNP, chronic rhinosinusitis with nasal polyposis; L-ASA, lysine-aspirin nasal challenge test; CT, computed tomography; RQLQ, rhinoconjunctivitis quality of life questionnaire; NO, nitric oxide; FEV₁, FEV₁ %, forced expiratory volume in 1 second % of predicted value; FEF₂₅₋₇₅, FEF₂₅₋₇₅, forced expiratory flow between 25% and 75% % of predicted value; sREFF, specific airways resistance % of predicted value; PC₂₀, provocative concentrations of methacholine required to produce a 20% drop in FEV₁.

A positive nasal L-ASA test was defined as a 25% decrease in total nasal volume using acoustic rhinometry.

75 participants (39 males) with a mean age (range) 51 (34-75) years completed the study. There were no allergic reactions or other serious adverse events during the course of the study.

23 (31%) participants gave a history of aspirin intolerance and 38 (51%) had a positive nasal L-ASA challenge according to acoustic rhinometry criteria and 30 (40%) with PNIF criteria. Test sensitivity was 48%, specificity was 52%, positive predictive value was 29% and negative predictive value was 68%.

Using the EACCI/GA2LEN 2007 criteria there was no difference in various baseline, upper and lower airway outcomes in patients with CRSwNP (with and without asthma) when comparing a history of aspirin tolerance versus intolerance.

Similarly there was no difference in the phenotypic characteristics of patients with or without a positive aspirin tolerance test (Table 9).

Table 10: Outcome comparison between groups.

Variable	CRSwNP		CRSwNP with asthma		<i>P</i>
	L-ASA positive	L-ASA negative	L-ASA positive	L-ASA negative	
No.	20	19	19	17	
Age—years	53±2	51±4	54±4	49±4	.44
Duration of rhinosinusitis—years	18±4	12±3	19±3	17±3	.45
Previous surgery—No.(%)	5 (25.7)	5 (26.3)	6 (31.6)	6 (35.3)	.92
CT score—units	18.5±3.0	24.9±2.5	27.3±4.1	22.5±6.8	.47
Polyp score—units	4.3±0.4	4.5±0.3	4.9±0.3	4.5±0.4	.56
Peak nasal inspiratory flow—L/min	140.6±13.7	94.7±8.8	114.1±12.6	109.1±12.8	.78
Total nasal volume—cm ³ *	33.8 (1.34)	45.39 (1.17)	22.39 (1.18)	41.69 (1.25)	.09
Total nasal symptoms	3.4±0.5	4.1±0.7	2.7±0.5	2.9±0.6	.82
Mini RQLQ—units	1.96±0.26	1.99±0.28	1.57±0.28	2.00±0.36	.32
Pocket Smell Test®—units	2.1±0.3	1.4±0.3	1.5±0.3	1.3±0.3	.70
Exhaled NO—ppb*	32.26 (1.16)	27.41 (1.21)	30.68 (1.27)	32.15 (1.22)	.87
Nasal NO—ppb*	387.25 (1.13)	400.19 (1.16)	389.10 (1.18)	402.22 (1.19)	.88
FEV ₁ —% predicted	104.3±3.2	98.8±3.3	82.4±4.2	86.8±3.0	.37
FEF ₂₅₋₇₅ —% predicted	90.9±5.8	86.3±5.7	60.2±5.2	61.9±5.8	.83
sReff— % predicted	73.6±7.2	81.4±5.2	139.5±35.9	126.9±12.9	.57
Methacholine PC ₂₀ —	32.0 (1.0)	24.5 (1.2)	2.9 (1.6)	1.9 (1.4)	.37
Serum IgE—kU/L*	86.31 (1.32)	69.72 (1.58)	142.55 (1.48)	133.11 (1.36)	.91
Eosinophil count—cells/uL*	0.31 (1.14)	0.31 (1.19)	0.35 (1.09)	0.44 (1.09)	.38

Plus-minus are arithmetic mean±SEM.

* Geometric mean (SEM).

Abbreviations: CRSwNP, chronic rhinosinusitis with nasal polyposis; L-ASA, lysine-aspirin nasal challenge test; CT, computed tomography; RQLQ, rhinoconjunctivitis quality of life questionnaire; NO, nitric oxide; FEV₁, FEV₁ %, forced expiratory volume in 1 second % of predicted value; FEF₂₅₋₇₅, FEF₂₅₋₇₅, forced expiratory flow between 25% and 75% % of predicted value; sREFF, specific airways resistance % of predicted value; PC₂₀, provocative concentrations of methacholine required to produce a 20% drop in FEV₁. A positive nasal L-ASA test was defined as a 25% decrease in total nasal volume using acoustic rhinometry.

Univariate analyses demonstrated a moderate association between aspirin challenge AUC and duration of rhinitis ($R=.35$, $P=.01$), $FEV_1\%$ ($R=.30$, $P=.04$) and $FEF_{25-75}\%$ ($R=.30$, $P=.03$). In univariate analysis, Mini RQLQ, smell test score, history of aspirin intolerance, FE_{NO} and sex approached statistical significance ($P<.1$ and $>.05$). These variables were therefore included with duration of rhinitis, $FEV_1\%$, $FEF_{25-75}\%$ and age in the regression model. In the multivariate regression analysis, after correction for other variables $FEF_{25-75}\%$ ($P=.004$), history of aspirin intolerance ($P=.013$) and duration of rhinitis ($P<.001$) were the only independent predictors of aspirin challenge AUC ($R=.59$ for the model).

Discussion

We aimed to characterize the phenotype of a cohort of adult patients with CRSwNP with or without asthma undergoing a nasal lysine-aspirin (L-ASA) challenge. In the present study, we have shown that patients with CRSwNP and a positive nasal L-ASA challenge do not have significantly more severe disease as compared to their test negative counterparts. We have also shown that a prolonged duration of disease and a history of aspirin intolerance highly correlate with a positive nasal L-ASA challenge test.

The EACCI/GA2LEN 2007 guidelines specify cut-off points for defining a positive nasal L-ASA test(102). When we evaluated patients with these criteria, they did not differ significantly in nasal, lower airway or quality of life outcomes (Table 9).

Indeed, the positive predictive value of this test in our cohort was only 29%. This indicates that using a cut-off point to define aspirin sensitivity may be a blunt instrument, especially when applied to a mixed population with regards to a history of aspirin intolerance. It has been suggested that history alone may overdiagnose aspirin sensitivity due to confounding factors and that ‘subclinical’ aspirin intolerance does not exist, thus explaining the need for formalised testing to diagnose this condition(283, 289). In our study, out of 52 patients without a history of aspirin intolerance, 27 had a positive test (52%). This is at odds with the findings of Killen and coworkers where apparently only 4 out of 32 patients with CRSwNP (with or without asthma) had a positive L-ASA test in the absence of historical aspirin intolerance(283). On closer evaluation, it is seen that the authors did not confirm the presence or grade of nasal polyps with endoscopy at initial selection. Moreover, they performed bronchial L-ASA testing; it may be that nasal response to aspirin precedes

the lower airways response. It is also possible that in our group, with an endoscopic confirmation of nasal polyps, a tighter diagnosis of asthma (including methacholine challenge and symptoms) and a history of surgery in 29% of patients, we had a cohort with greater disease severity. Our prevalence of aspirin sensitivity with clinical history and nasal L-ASA test is generally in agreement with the literature(95). Thus, in our population there were many patients with a positive L-ASA test without overt symptoms or without severe CRSwNP bringing into focus the possible lack of clinical correlation of this test.

Our multivariate regression analysis demonstrated that FEF₂₅₋₇₅, history of aspirin intolerance and duration of rhinosinusitis were the only significant predictors of response to nasal L-ASA. FEF₂₅₋₇₅ is a well-known predictor of small airways disease in patients with rhinitis and may be an early marker of lower airway dysfunction(290). Both FEV₁ and FEF₂₅₋₇₅ have been seen to fall at 4-year follow-up in treatment-resistant CRSwNP whereas treatment-responsive patients remained stable (291). The role of FEF₂₅₋₇₅ in CRSwNP and AIR remains to be defined. It is well known that AIR does not occur randomly in CRSwNP and asthma. It is believed that the onset of AIR starts in early life, progressing through the stages of rhinitis and finally attaining full clinical expression in the fourth decade of life(35). Perhaps, longitudinal community-based studies could investigate the role of FEF₂₅₋₇₅ in patients who subsequently develop AIR, similar to allergic rhinitis. That the duration of rhinosinusitis symptoms and a history of aspirin sensitivity correlate with a nasal L-ASA challenge is unsurprising, given the pathogenesis of the disease. This is similar to the findings of Hope and colleagues where they demonstrated an increased frequency of more severe reactions to oral aspirin in patients with a history

of aspirin sensitivity and reduced lung function(292). It is well known that aspirin intolerance is a significant predictor of severe recalcitrant disease and is associated with greater exacerbations and poorer response to treatment than aspirin tolerant patients(35, 99).

Current guidelines recommend greater use of aspirin challenge testing in the diagnosis of AIR, but fail to mention which population these tests are to be applied. Killen and coworkers suggested that in the absence of a positive clinical history, L-ASA challenge testing was not likely to be positive(283). Our results would also not support the use of nasal L-ASA challenge test with single cut-off points as a screening tool in patients with rhinosinusitis as a whole. This is so, because it is unclear what the clinical significance of a positive test in patients without a history of clear reactions to aspirin or NSAIDs is, as clearly this test fails to select out a more severe phenotype of CRSwNP with/without asthma. It is to be borne in mind that ours is a single snapshot view, and that such patients may over time go on to develop severe symptoms and exacerbations. In the absence of prospective, longitudinal data, we accept that one cannot rule out the yield or usefulness of this test. Perhaps, in well-selected patients who have severe symptoms, long duration of illness, with a verbal history of aspirin intolerance, and associated spirometric abnormalities this test yield may go up. Further, at present there is a paucity of randomized placebo controlled studies looking at definitive treatment options like aspirin desensitization(9, 102). It is difficult at present to recommend a test without a clear treatment protocol to follow a positive test, especially if patients are asymptomatic.

We haven't performed oral aspirin challenge tests in our patients.

Milewski et al.(293) and Alonso-Llamazares et al.(294) found that the specificity of nasal challenge with lysine- aspirin reached 95.7% and 92.5% respectively, whereas its sensitivity was 86.7% and 80%, respectively. The predictive value of a negative result of l-ASA nasal challenge was 78.6% and 89.2%, respectively. More recently, Gonzalez-Perez et al. showed sensitivity, 87%; specificity, 100%; positive predictive value, 100%; negative predictive value, 86%(286). These show that nasal L-ASA tests have comparable test characteristics to oral aspirin challenge testing. Moreover, they are safer with regard to the likelihood of allergic and anaphylactic reactions(102).

In our experience of treating patients with chronic rhinosinusitis and asthma for over a decade, we have most commonly seen clinicians rely on a history of aspirin sensitivity rather than offer a provocation test it in a general group of CRS patients. While this may be said to underdiagnose patients with ‘aspirin sensitivity’ in that there are likely to be undiagnosed patients who may have a positive challenge test, routine testing in an unselected group of patients with CRS may be of very low yield. Moreover, the significance of a positive test in an individual with mild-moderate CRS (our cohort) is unclear, i.e. would such patients benefit from desensitization?

Our study was not designed to look at the test characteristics of nasal aspirin provocation testing vis-à-vis oral provocation testing. Instead, we studied an unselected cohort of patients with CRS with and without asthma to see if a positive nasal aspirin test would predict a more severe disease phenotype. We believe that our patients reflect a large majority of CRS in terms of disease severity, and we wish to question the wisdom of routine aspirin sensitivity testing as proposed by the 2007 EACCI/GA2LEN guidelines(102).

In summary, we showed in a cohort of CRSwNP with and without asthma that a positive nasal aspirin challenge test does not predict a more severe disease phenotype. Factors associated with a positive nasal aspirin challenge include a history of aspirin intolerance, duration of rhinitis and FEF₂₅₋₇₅.

Chapter 6: To optimise and extend the duration of use of nasal sympathomimetic decongestants by evaluating tachyphylaxis of response to nasal oxymetazoline and its reversal by fluticasone

Study Aims – Using a double-blind randomized placebo-controlled crossover design to

1. Establish if tachyphylaxis and rebound congestion occurs after use of oxymetazoline nasal spray.
2. Demonstrate if reversal of effect can be achieved by intranasal fluticasone propionate.

3. Dissect out the relative α_1 -/ α_2 -adrenoceptor components of tachyphylaxis using the α_1 -antagonist prazosin.

Introduction

Alpha-adrenoceptor agonists, in particular, imidazoline derivatives like oxymetazoline are the most efficacious medications available for the acute relief of nasal congestion in chronic rhinosinusitis(2). It is held that prolonged use is heralded by a reduction in efficacy (tachyphylaxis), as well as a rebound increase in nasal airway congestion and non-specific nasal hyper-reactivity, which have been combined under the umbrella term of ‘rhinitis medicamentosa’(118). The *in vitro* regulation of adrenoceptors by agonists has been extensively studied and is characterized by a combination of rapid internalization and uncoupling, with delayed degradation and sub-sensitivity(111). However, the clinical onset of tachyphylaxis and rebound congestion in patients remains controversial. A randomized double blind controlled trial by *Morris et al* showed an increase in baseline nasal airway resistance after 3 days of treatment with oxymetazoline in healthy volunteers(295). A series of clinical trials by *Graf et al* have shown that 4 weeks of treatment with oxymetazoline causes nasal congestion and hyper-reactivity, an effect aggravated by the preservative benzalkonium chloride(296-298). Conversely, in a randomized clinical trial in healthy volunteers, *Watanabe et al* did not demonstrate any change in subjective or objective outcomes after 4 weeks of oxymetazoline(299). The onset, mechanism and modulation of alpha-adrenoceptor tolerance in the nose are poorly understood. Despite an absence of definitive evidence, international guidelines restrict the use of topical decongestants in rhinitis to less than 10 days, limiting their potential role as a treatment(2).

While various studies have investigated the putative role of intranasal corticosteroids in the treatment of decongestant overuse, it is difficult to establish true efficacy due to underlying pre-existing rhinitis(118). For example, in a randomized clinical trial by *Ferguson et al*, 20 treatment naïve subjects with perennial allergic rhinitis were randomized to receive budesonide nasal spray or placebo after 2 weeks of oxymetazoline nasal spray(300). Unsurprisingly, participants receiving budesonide experienced greater symptomatic relief than those on placebo. Crucially, the onset of tolerance was not demonstrated and one could only conclude that corticosteroids were beneficial in treating allergic rhinitis. In contrast, beta-adrenoceptor tolerance with prolonged agonist use has been comprehensively investigated in the lower airways(301). We have previously demonstrated that systemic corticosteroid treatment acutely reverses formoterol induced bronchodilator sub-sensitivity and beta-adrenoceptor down-regulation in asthmatics within 1 hour of administration (138).

Lastly, in an *in vitro* study it has been suggested that nasal decongestion occurs mainly at the deep venous sinusoids via α_2 -adrenoceptor(116). On the other hand, intense arteriolar vasoconstriction at the α_1 -adrenoceptors has been implicated for side effects such as rebound congestion, purportedly due to mucosal ischemia(302). Thus, it is theorized that a selective α_2 -agonist (which is not presently available) may have an improved safety profile(116, 303).

We have dissected out α_1 -/ α_2 -adrenoceptor mediated components of tachyphylaxis and associated rebound congestion with oxymetazoline (a mixed α_1 -/ α_2 -agonist), by using the selective α_1 -antagonist prazosin. We have also evaluated if reversal of

tachyphylaxis and associated rebound congestion occurs with the concomitant use of an intranasal corticosteroid as fluticasone propionate. We used peak nasal inspiratory flow and nasal airway resistance to measure nasal airway congestion (as surrogates of venous sinusoidal filling) and mucosal laser Doppler flowmetry as a measure of superficial arteriolar flow.

Methods

The Tayside committee for medical research ethics gave institutional approval to the study protocol before commencement of the trial, and written informed consent was obtained from each participant. All authors had full access to the data and manuscript content and contributed to the data analysis. The trial was registered on www.clinicaltrials.gov, identifier number NCT00487032.

PARTICIPANTS

Using our research volunteer database, we recruited healthy adults, none of whom were smokers. Inclusion criteria were: male or female healthy volunteers, aged 18 to 65 years, without nasal or ocular symptoms suggestive of rhinitis or rhinosinusitis as per the 2008 ARIA(2) and 2007 EPOS(13) guidelines respectively, negative skin prick test to common aeroallergens, normal ECG, normal blood pressure with no postural hypotension, peak nasal inspiratory flow rate (PNIF) $> 100\text{L}\cdot\text{min}^{-1}$ (best effort of three), and PNIF reversibility with oxymetazoline 0.05% w/v 2 squirts in each nostril (20 min reading) $> 20\text{L}\cdot\text{min}^{-1}$. Exclusion criteria were: a history of sympathomimetic decongestants or alpha blockers in the previous 6 months, recent or concomitant upper respiratory tract infection, mechanical nasal airway obstruction due to septal deviation greater than 50% at nasoendoscopy, pregnancy, lactation or any medical condition or screening blood result likely to compromise participant safety.

STUDY DESIGN

In this randomized, double-blind, placebo-controlled, cross-over design study, participants attended the research unit 6 times between July 2008 and July 2009, in Dundee, Scotland (Figure 10). Following a 1 week run-in, participants received oxymetazoline hydrochloride nasal spray (0.05% w/v) 2 squirts in each nostril (200 µg) 3 times daily (on waking up, after lunchtime and before going to bed) for 14 days. For the next 3 days, in addition to this dose of oxymetazoline, participants were given fluticasone propionate nasal spray, 2 squirts in each nostril (200µg) twice daily for a further 3 days, making the total length of each treatment period 17 days. At each visit, after the baseline efficacy and safety measurements were taken, a single dose of prazosin hydrochloride 1mg or matching placebo was administered orally with participants in a supine position. For each treatment period, prazosin (a selective α 1-antagonist) or placebo was administered in a randomised assignment order i.e. participants were given prazosin for the first 3 visits followed by placebo or vice versa. A week's washout separated each treatment period.

MEASUREMENTS

At screening, inclusion and exclusion status was determined as per above. All participants were issued with a PNIF and symptom diary. Each visit started between 8 am and 9 am and measurements were taken at a room temperature of 21–23°C and at constant relative humidity after a 20 minute acclimatization period. No caffeine containing drinks were permitted in the preceding 2 hours and alcohol for 24 hours. At the first treatment visit, baseline values for all primary and secondary efficacy, and safety outcomes were established. PNIF (primary outcome), and total nasal airway resistance (NAR) with active anterior rhinomanometry were used as measures of nasal airways patency. The PNIF measurements were taken as the best

of 3 measures from an In-check flow meter (Clement Clarke International Ltd, Harlow, England). Technique was evaluated to ensure a seated posture, horizontal positioning of the meter, correct restoration of the reading to zero, a closed mouth, and an adequate mask seal while making a maximal nasal inspiration. NAR using active anterior rhinomanometry was measured at 150 Pa using an NR6 rhinomanometer (GM instruments, Kilwinning, UK) according to the recommendations of the Standardisation Committee on Objective Assessment of the Nasal Airway, I.R.S. and E.R.S.(221) In order to reflect the adrenoceptor status on the arterioles, single point laser Doppler flowmetry (LDF) was employed using a custom built PF-5 needle probe attached to the MBF3D™ monitoring system (Moor Instruments, UK) to estimate mucosal blood flow(210). The probe was positioned on the nasal mucosa of the inferior turbinate (the position marked and used at each subsequent visit) using a 2.7 mm 30° endoscope (Karl Storz-Endoskope; Tuttlingen, Germany). Horizontal head position and immobilisation was achieved with a custom-made head strap and chin-stabilizer. A micromanipulator with an *x-y-z* axis translator was used for fine adjustment of probe position. An average of 3 minutes of reading was taken after an initial stable tracing was achieved.

2 hours after the administration of prazosin or placebo, a dose-response curve was constructed using doubling doses (sum of both nostrils) of oxymetazoline of 25µg, 50µg, 100µg and 200µg at 20 minute intervals. PNIF was measured at baseline and after each successive dose. As safety outcomes, lying/standing blood pressure (BP) and heart rate was measured at half-hourly intervals for the first two hours and hourly thereafter until either 4 hours had elapsed or no postural drop in blood pressure (or reflex tachycardia) was recorded, whichever came first. Index finger-tip

blood flow using the laser Doppler flowmeter was measured at baseline and 2 hours after prazosin or placebo as an estimate of systemic alpha blockade(304).

Participants were sent home with instructions to use the oxymetazoline at the abovementioned doses and canister weights were measured at each visit to assess compliance. They also recorded domiciliary PNIF and a nasal blockage score on a scale of 0-3 before the evening dose to assess any rebound nasal congestion at trough. Participants withheld their previous night's oxymetazoline prior to their visit.

RANDOMIZATION AND BLINDING

An independent off-site clinical trials pharmacist (Pharmacy Production Unit, Western Infirmary, Glasgow, UK) used a computer-generated random allocation sequence to randomize the trial. The same pharmacist masked and blinded the prazosin 1mg tablet and an identical placebo tablet to double-blind the study from the investigator and participants. These were administered using sealed opaque envelopes at the research unit.

STATISTICAL ANALYSIS

The study was powered at > 80 % with an alpha-error of 0.05 (two-tailed) in order to detect a $10\text{L}\cdot\text{min}^{-1}$ difference in PNIF between randomised treatments, with an estimated sample size of 16 participants assuming the within-subject SD to be $9.2\text{L}\cdot\text{min}^{-1}$ (205). This was considered adequate to demonstrate tolerance to oxymetazoline i.e. change in PNIF within each subject, considering previous literature(298, 305). Each outcome was assessed for normality using the Shapiro-Wilk test and by visual inspection of histograms and Q-Q plots, with consideration

of previous datasets and literature. Non-normal data were logarithmically transformed. An overall analysis of variance was performed with subject, treatment and sequence as cofactors followed by Bonferroni-corrected pair-wise comparisons with a 2-tailed α -error set at 0.05. The dose-response curves was analyzed using a two-way ANOVA factoring in time in addition to the above mentioned factors, in order to obviate multiple comparisons at several time points. All analyses were performed on a per-protocol basis using SPSS version 17, Chicago, IL, USA.

Results

PARTICIPANTS

Of the 33 patients screened, 25 underwent randomization to a treatment group. 6 participants were withdrawn: 3 could not complete for personal reasons, 1 withdrew after an episode of vasovagal syncope prior to administration of prazosin, 2 because of non-compliance with the requirements of the protocol. 12 female and 7 male participants with a mean (range) age of 33 (21-55) years completed per protocol. There were no serious adverse events. 2 participants had dizziness on standing up after taking prazosin, but this resolved spontaneously.

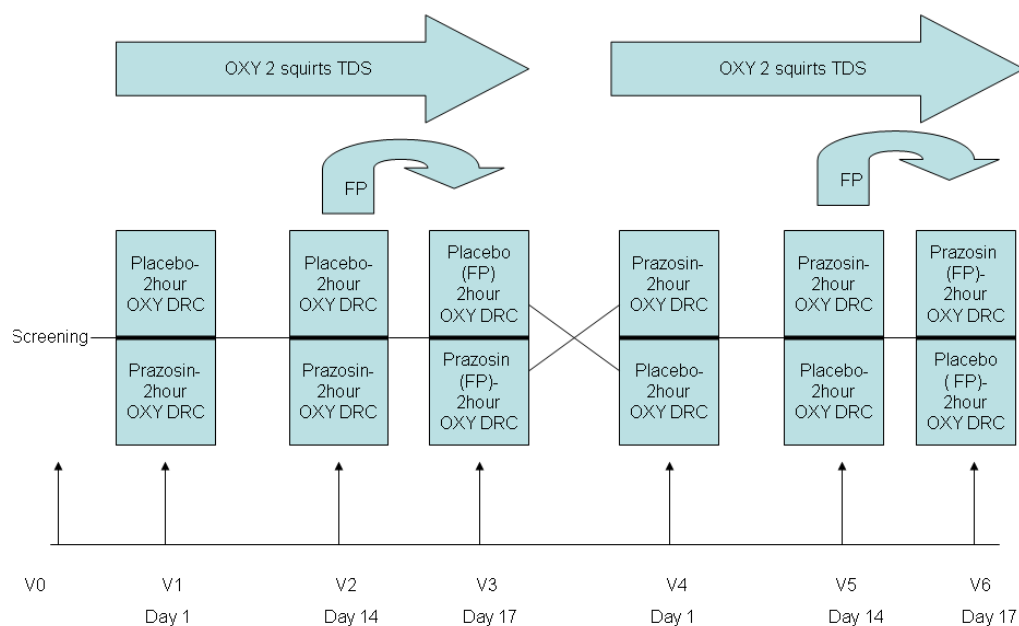


Figure 13: Study design.

V0 is screening visit, V1-V6 are study visits. Abbreviations: OXY is oxymetazoline 200 μ g TDS from Day 1 till Day 17; DRC is Oxymetazoline dose response curve estimation (25 μ g, 50 μ g, 100 μ g, 200 μ g) 2 hours after administration of oral prazosin or placebo; FP refers

to Fluticasone aqueous nasal spray started as add-on to OXY from Day 14 till Day 17.

BASELINES

For all outcomes, there were no significant differences between the first and second baseline visits in sequence after run-in and washout, respectively; or between the baselines prior to each respective treatment arm, irrespective of sequence.

Table 11: Comparisons of baselines.

	Baselines according to sequence			Baselines according to treatment		
	Baseline 1	Baseline 2	P	Baseline 1	Baseline 2	P
	Mean (95% CI)	Mean (95% CI)		Mean (95% CI)	Mean (95% CI)	
PNIF (L.min ⁻¹)	185.3 (166.4,204.1)	190.0 (168.5,211.4)	.74	194.7 (173.7,215.7)	180.5 (161.7,199.3)	.32
NAR (Pa.s.cm ⁻³)	0.31 (0.25,0.36)	0.25 (0.21,0.29)	.08	0.29 (0.25,0.33)	0.25 (0.20,0.30)	.26
LDF (units)	287.0 (255.1,319.0)	299.0 (259.5,338.5)	.64	298.2 (262.6,333.9)	288.4 (252.0,329.8)	.70

Abbreviations: PNIF, Peak Nasal Inspiratory Flow Rate; NAR, Nasal Airway Resistance; LDF, Laser Doppler Flowmetry; CI, Confidence Intervals
 *Data are arithmetic mean (95% Confidence Intervals)

Thus outcomes prior to oxymetazoline dose-response challenge were therefore pooled for the purpose of subsequent analyses.

CHANGE IN NASAL AIRWAY PATENCY WITH CHRONIC DOSING

The effects of chronic dosing with oxymetazoline on PNIF and NAR are shown in Figure 15.

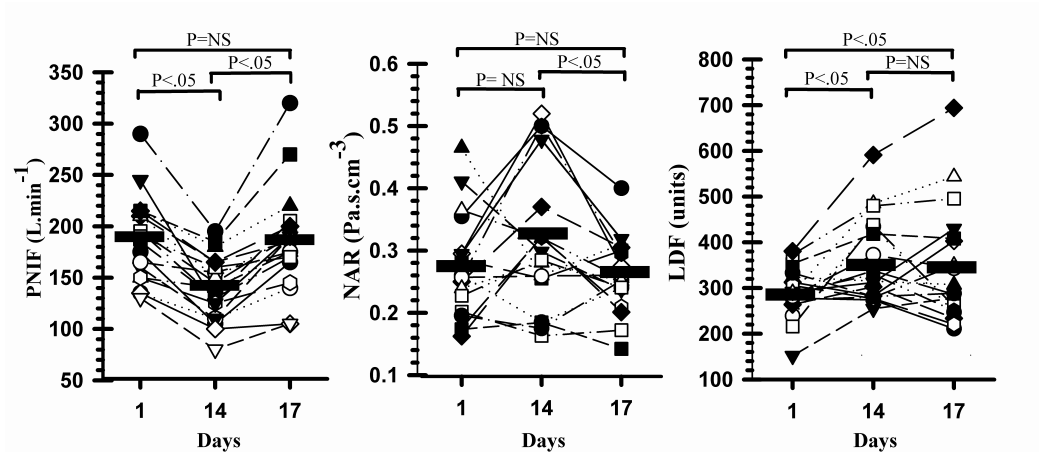


Figure 14: Outcomes before and after chronic dosing with oxymetazoline.

Effect of oxymetazoline on peak nasal inspiratory flow rate (PNIF), nasal airway inspiratory resistance (NAR) and laser Doppler mucosal blood flow (LDF), and reversal with fluticasone. Symbols represent individual patients and pooled values for both treatment periods. Horizontal bars represent arithmetic means. Measurements were taken at baseline (Day 1), after oxymetazoline 200µg tid (Day 14), and addition of fluticasone 200µg bid for 3 days (Day17).

Visit-based PNIF decreased at Day 14 compared to Day 1 as mean difference (95% CI, P): -47.9 L.min^{-1} (-63.92 to -31.87 , $P < .001$). NAR increased by $0.05 \text{ Pa.s.cm}^{-3}$ (0.004 to 0.11 , $P = .12$).

CHANGE IN NASAL MUCOSAL BLOOD FLOW WITH CHRONIC DOSING

The effect of chronic oxymetazoline dosing on nasal mucosal blood flow as measured by laser Doppler flowmetry is shown in Figure 16. Nasal blood flow increased by 65.4 units (10.7 to 120.1, $P = 0.01$) on Day 14 compared to Day 1.

EFFECT OF PRAZOSIN ON OUTCOMES

Effect of 1mg oral prazosin and placebo on PNIF, NAR, nasal blood flow and fingertip blood flow (positive control) is shown in Figure 16, Figure 17 and Table 11.

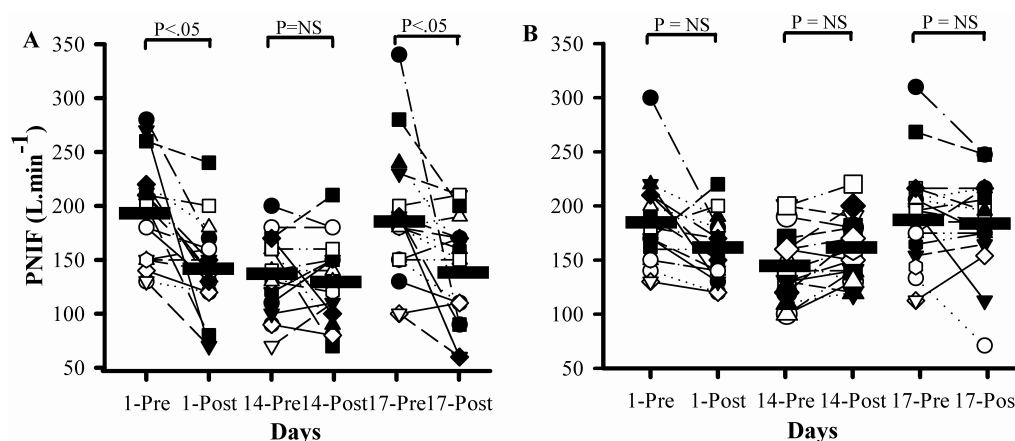


Figure 15: Effect of prazosin and placebo at baseline, post oxymetazoline, and post fluticasone.

Effect of 1mg oral prazosin (Panel A) and placebo (Panel B) on peak nasal inspiratory flow rate (PNIF) at baseline (Day 1), after oxymetazoline 200 μ g tid (Day 14) and after the addition of fluticasone 200 μ g bid for 3 days (Day 17).

Measurements were made before and 2 hours after prazosin was administered.

Horizontal bars are arithmetic means. Effect of prazosin on PNIF (Day 1) due to alpha-1 adrenoceptor blockade is masked due to down-regulation after chronic

dosing of oxymetazoline (Day14). Fluticasone up-regulates the alpha-1 adrenoceptors and ‘restores’ the congestive effect of prazosin (Day17).

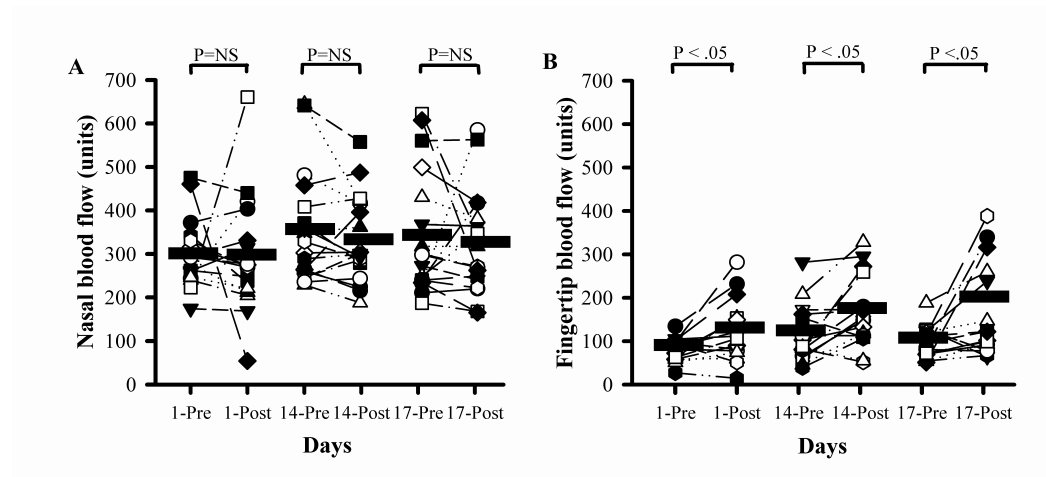


Figure 16: Effect of prazosin on nasal and skin blood flow.

Effect of 1 mg oral prazosin on nasal mucosal blood flow (Panel A) and fingertip blood flow (Panel B) at baseline (Day 1), after oxymetazoline 200 μ g tid (Day 14) and addition of fluticasone 200 μ g bid for 3 days (Day17). Measurements were made before and 2 hours after prazosin was administered. Horizontal bars represent arithmetic means. Prazosin had no significant effect on nasal blood flow but increased fingertip blood flow (positive control).

Table 12: Effect of prazosin and placebo on nasal patency and mucosal blood flow.

Variable	Prazosin			Placebo		
	Pre	Post	P	Pre	Post	P
PNIF (L.min ⁻¹)						
Day1	194.7(172.8–216.7)	142.1(121.9–162.3)	<.001	180.5(160.9–200.2)	165.8(145.1–186.4)	.28
Day14	137.4(120.4–154.4)	129.5(110.8–148.1)	.51	142.1(126.2–158.0)	143.2(127.9–159.4)	.88
Day17	186.8(159.1–214.6)	138.4(113.9–162.9)	.009	182.6(160.1–205.2)	173.7(152.5–194.9)	.55
NAR (Pa.s.cm ⁻³)						
Day1	0.25(0.20–0.30)	0.35(0.28–0.43)	.03	0.29(0.25–0.33)	0.38(0.25–0.51)	.17
Day14	0.32(0.23–0.40)	0.52(0.36–0.69)	.03	0.33(0.26–0.40)	0.32(0.25–0.38)	.79
Day17	0.26(0.22–0.29)	0.60(0.28–0.92)	.03	0.26(0.22–0.29)	0.27(0.25–0.31)	.61
Nasal blood flow (units)						
Day1	288.4(250.4–326.4)	325.3(276.2–374.5)	.23	282.5(235.6–329.4)	279.9(213.7–346.0)	.95
Day14	361.9(293.4–430.0)	386.8(336.0–437.6)	.50	354.3(296.4–412.2)	330.8(285.8–375.8)	.52
Day17	357.4(276.3–438.5)	395.7(295.1–496.4)	.57	340.9(277.0–404.7)	324.9(271.1–378.6)	.70

Data are arithmetic means (95% CI). P values are derived from an overall ANOVA with Bonferroni corrected multiple range testing. Abbreviations: PNIF, Peak Nasal Inspiratory Flow Rate; NAR, total nasal inspiratory resistance. Measurements were made before and 2 hours after 1mg oral prazosin or identical placebo was administered. Day 1 is baseline, Day 14 is after chronic oxymetazoline dosing 200µg tid, and Day 17 is after the addition of intranasal fluticasone 200µg bd.

OXYMETAZOLINE DOSE RESPONSE

The oxymetazoline DRC before and after chronic dosing with oxymetazoline 200µg tid, and after the addition of intranasal fluticasone is shown in Figure 18.

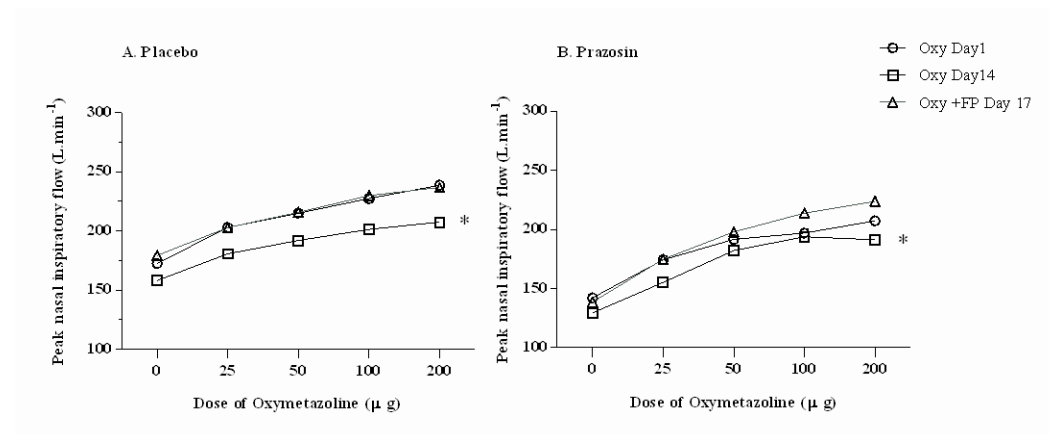


Figure 17: Oxymetazoline dose response curves at baseline, post chronic oxymetazoline dosing and post fluticasone.

Oxymetazoline dose-response curves (DRC) for peak nasal inspiratory flow at baseline (Day 1), after oxymetazoline 200µg tid (Day 14) and addition of fluticasone 200µg bid for 3 days (Day17). Symbols represent mean and SEM. * represents a significant ($P < .05$) difference between baseline (Day 1) and chronic dosing (Day 14) with oxymetazoline.

The DRC (as absolute PNIF values) after either administration of placebo or prazosin showed dose-dependent increases in PNIF, and a plateau in response was not attained at the end of the dose range, at $t = 80$ min. There was a downward parallel shift of the PNIF DRC after chronic oxymetazoline dosing, after placebo (24.8 L.min^{-1} ; 20.3 to 29.3, $P < .001$) and prazosin (12.0 L.min^{-1} ; 1.1 to 22.9, $P < .05$).

EFFECTS OF INTRANASAL STEROID

As depicted in Figure 16, at Day 17 compared to Day 1, administration of intranasal fluticasone reversed the reduction in PNIF (-2.9 L.min^{-1} ; -18.9 to 13.1) and increase in NAR ($-0.01 \text{ Pa.s.cm}^{-3}$; -0.06 to 0.05) to baseline levels, but did not influence nasal mucosal blood flow (60.2 units; -5.5 to -114.9). There was significant reversal of PNIF DRC tachyphylaxis on Day 17 (Figure 18), evidenced by an upward and parallel shift of the curve as compared to Day 14 after placebo (26.2 L.min^{-1} ; 21.7 to 30.7 , $P < .001$) and prazosin (19.3 L.min^{-1} ; 8.4 to 30.2 , $P < .01$).

DOMICILIARY MEASURES

There was a significant increase in the mean nasal blockage score on Day 14 vs. Day 1 (0.45 units; 0.10 to 0.79), with a non-significant reduction after fluticasone on Day 17 vs. Day 14 (-0.29 units; -0.64 to 0.06). Evening PNIF decreased non-significantly at Day 14 vs. Day 1 (-7.4 L.min^{-1} ; -40.1 to 25.4), but increase significantly after fluticasone on Day 17 vs. Day 14 (36.6 L.min^{-1} ; 3.9 to 69.3).

CARDIOVASCULAR OUTCOMES

The mean standing blood pressure fell ($P < .05$) after the administration of prazosin with a peak fall at $t = 120$ minutes (see Figure 19). The mean heart rate increased after prazosin to a peak rise at $t = 90$ minutes (see Figure 20). The time profile curve for standing mean arterial pressure and standing heart rate was significantly different for prazosin compared to placebo as mean difference (95% CI): -3.2 mmHg (-6.4 to -0.1) and 11 bpm (5 to 18).

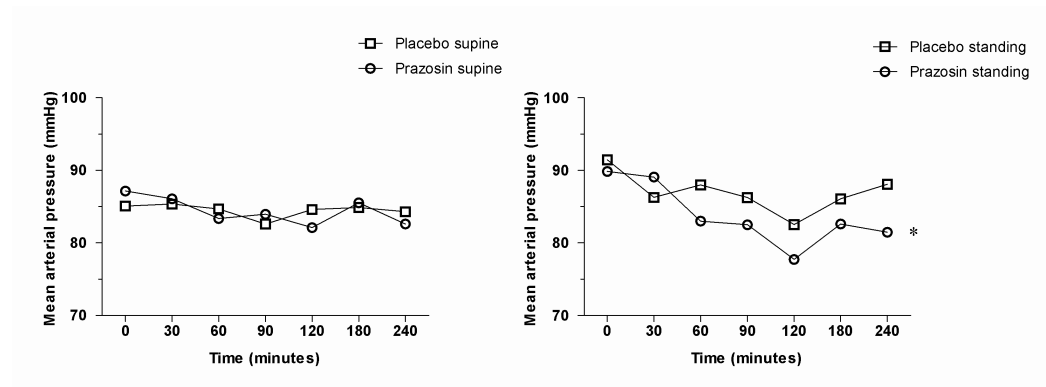


Figure 18: Time-profile curve for mean arterial pressure after prazosin 1mg vs. placebo.

Supine and standing mean arterial pressure time profile before ($t = 0$) and after 1mg of prazosin or placebo. Symbols denote mean pooled values for all visits. * denotes a significant mean difference ($P < .05$) between the prazosin and placebo arms.

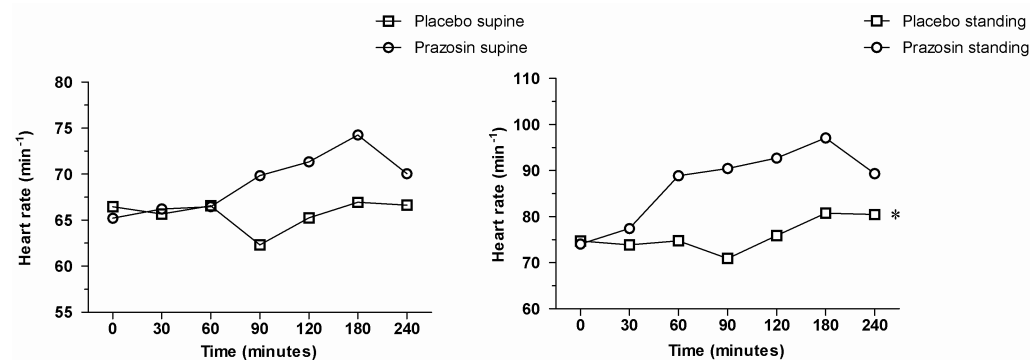


Figure 19: Time-profile curve for heart rate after prazosin 1mg vs. placebo.

Serial heart rate measurements before ($t = 0$) and after 1mg oral prazosin or placebo.

Symbols denote mean pooled values for all visits. * denotes a significant mean difference ($P < .05$) between the prazosin and placebo arms.

Discussion

The results of the present study demonstrate that tachyphylaxis of response to oxymetazoline and rebound nasal congestion occurs after 14 days of treatment. The relative α_1/α_2 components to sub-sensitivity were ascertained from the nasal patency

measures and oxymetazoline dose response curve pre- and post-prazosin. We also demonstrated for the first time, reversal of tachyphylaxis with intranasal corticosteroid. Our data suggest that the nasal congestive response is predominantly mediated by $\alpha 1$ -adrenoceptors.

We chose to conduct the present study in healthy subjects to obviate any confounding nasal congestion due to pre-existing allergic or non-allergic nasal disease. We hypothesized that decongestant sub-sensitivity was primarily due to receptor down-regulation, and could be demonstrated on healthy nasal mucosa. We used oxymetazoline at its maximum recommended dose of 200 μ g tid to ensure maximal occupancy of nasal adrenoceptors and withholding the previous night's dose was adequate to washout the drug(306). The doses of oxymetazoline used to construct the DRC were estimated from previous pilot studies (unpublished) conducted by our department and from literature which showed that they would represent the steep portion of the curve for PNIF(307). The dosing intervals were selected so as to ensure maximal decongestion at each dose within time constraints of the overall DRC(306). Finally, we constructed the DRC 2 hours after prazosin or placebo to reflect time of peak prazosin plasma concentration and peak alpha1 receptor blockade(304, 308).

We demonstrated a statistically and clinically significant reduction in PNIF (Figure 15), but not in NAR, after 14 days of oxymetazoline with a mean reduction of 47 L.min⁻¹ (7 times the minimal important difference)(271). This rebound congestion is most likely a result of receptor down-regulation and uncoupling, indirectly influencing the basal sympathetic tone of the mucosal sinusoids i.e. by decreasing

sensitivity to endogenous circulating catecholamines at trough(309). We also showed tachyphylaxis to the decongestant response by a downward parallel shift of the PNIF DRC after 14 days of oxymetazoline (Figure 18). These findings are in keeping with the majority of literature in alpha-agonist use in healthy volunteers(209). We believe that the lack of effect using rhinomanometry can be explained by its high within-subject variability as compared to PNIF and that it is a measure probably more suited to short-term intra-individual measurements, such as in a nasal challenge(205). We also ensured a priori that our participants were on the top of the learning curve for PNIF by sending them away with a PNIF diary prior to randomization.

We were able to estimate the differential effects of oxymetazoline and receptor tolerance on the α_1 - and α_2 -adrenoceptors by using prazosin, a selective α_1 -antagonist(113). A single dose of prazosin reduced mean PNIF by 50 L.min⁻¹ and shifted the decongestive dose-response to the right (Figure 15 and Figure 18). Our study is the first clinical trial to document the acute congestive effect of prazosin objectively, a phenomenon that has only been anecdotally observed for the last 4 decades, since the inception of its use.

Oxymetazoline, a noradrenaline analogue is a mixed alpha-adrenoceptor agonist with predominant action at the α_2 adrenoceptor and an α_2 : α_1 potency ratio of 5:1(113). Under resting conditions (absence of ligand), endogenous circulating catecholamines act on nasal α -adrenoceptors and keep the deep venous sinusoids under a state of dynamic venous contraction, which manifests as a decongested nose(110). Under the influence of a ligand like chronic oxymetazoline, rapid

receptor internalization followed by uncoupling occurs(309) and the sinusoids ‘dilate’ leading to congestion (decrease in PNIF). Likewise, in the current study, chronic dosing with oxymetazoline resulted in tachyphylaxis at the deep venous sinusoidal adrenoceptors, an effect manifested by a reduction in PNIF on Day 14 (Figure 15). Moreover, the congestive effect of a single dose of the α_1 -antagonist prazosin seen on Day 1 is lost on Day 14 (Figure 16). This suggests that the predominant adrenoceptor in the venous sinusoids is α_1 and also that tachyphylaxis occurs predominantly at the α_1 -adrenoceptor. This seeming paradox, whereby despite using a predominant α_2 -agonist, the tachyphylaxis is observed at the α_1 -adrenoreceptor, can only be explained if the predominant functioning receptor subtype in the sinusoids is α_1 . This also indicates that the resting sympathetic tone in the deep venous sinusoids is predominantly α_1 mediated. This is in keeping with the findings of *Ichimura et al*, who found that while the human nasal mucosa has both post-junctional α_1 and α_2 adrenoceptors, the α_1 mediated contractile response was larger(119). This contrasts with the *ex vivo* findings of *Corboz et al* and highlights the pitfalls of making conclusions about functionality using tissue isolates(116). It is important to note that we showed tolerance at the α_2 receptor as well, with the downward parallel shift of the PNIF DRC post-prazosin (Figure 18).

In our study, prazosin had no effect ($P = .23$) on nasal mucosal blood flow as measured by laser Doppler flowmetry (Figure 17). This is in contrast to the effect of prazosin on fingertip blood flow, where it caused a significant increase. It has been previously shown that fingertip blood flow is tightly regulated by α_1 -adrenoceptors(304). It is also known that the predominant adrenoceptor type in the nasal arterioles and pre-capillary sphincter is α_1 (116, 117). Moreover, chronic

oxymetazoline dosing resulted in an increased mucosal blood flow (Figure 10).

Laser Doppler flowmetry of the nasal mucosa is thought to measure blood flow in the superficial capillary bed, arterioles and the copious arterio-venous shunts(210).

One plausible hypothesis is that the blood flow measured by laser Doppler flowmetry may be primarily α_2 mediated, and that the increase with chronic oxymetazoline is due to tachyphylaxis. Another likely explanation could be that mucosal blood flow might be at a near maximum and hence was not influenced by prazosin. Indeed, the magnitude of nasal blood flow is almost 3 times as compared to fingertip blood flow (Figure 17), which also has numerous arterio-venous shunts. This disconnect between the superficial mucosal blood flow and the deep venous sinusoidal response to prazosin, could exist to keep the nasal mucosa perfused despite fluctuations in nasal blood volume occurring many times a day as part of the nasal cycle.

A noteworthy fact is that the effect of chronic oxymetazoline dosing and acute prazosin dosing on the baseline PNIF (Figures 15,16) and the PNIF DRC (Figure 18) was similar. The PNIF DRC demonstrated a downward parallel shift, rather than a downward and rightward shift after chronic oxymetazoline dosing. To establish a rightward shift, an increase in ED50 would have to be demonstrated. We did not calculate the ED50 in this study, as we could not be certain we had established the maximal response to oxymetazoline. We used oxymetazoline at clinically recommended doses and measured the maximum elicitable response in PNIF at such doses. As such, whether this 'maximum' represents the maximal response to the ligand cannot be determined as the doses of oxymetazoline needed to establish this

would be outwith the recommendations of the British National Formulary guidelines and ethically challenging. Moreover, PNIF demonstrates a ceiling effect(310), in that it tends to plateau off despite continued increase in nasal airway patency, as it is limited by factors such as effort and baseline pulmonary function. However, the observed reduction in the maximal elicitable response at clinically recommended dosages is consistent with receptor internalization and G-protein- α -adrenoceptor uncoupling at the molecular level. A depletion of the receptor reserve would lead to a reduction of the maximum response rather than a rightward shift(311).

Intranasal corticosteroid as fluticasone at the maximum recommended dose of 200ug bid was highly effective in reversing the rebound congestion and PNIF increased back to baseline levels. Additionally, receptor sub-sensitivity was reversed as evidenced by the upward parallel shift in the PNIF DRC (Figure 16 and Figure 18). However, we could not demonstrate an effect of corticosteroid on mucosal blood flow. This may indicate that corticosteroids might reverse tolerance at the α 1- but not α 2-adrenoceptors, in the short term at least. Data on nasal α -adrenoceptor regulation is conspicuously absent in the rhinology literature. Likewise, while there is some data on α -adrenoceptor downregulation under the influence of agonists *in vitro* and *in vivo*, the regulatory mechanisms underpinning receptor expression and up-regulation, evaluation of the heterologous influences through interaction with other receptors, and subtype or tissue specific regulation are yet to be fully elucidated(309). Data from β -adrenoceptors suggests that corticosteroids acting through glucocorticoid response elements, restore G-protein- β 2-adrenoceptor coupling, increasing cell surface receptor numbers and inhibit and reverse β 2-adrenoceptor downregulation(312).

In spite of the many similarities between the structure and behaviour of α and β -adrenoceptors under agonist influence, an extrapolation of the mechanisms of regulation from one to the other may be too simplistic(313). Nonetheless, it may be hypothesized that corticosteroids influence α -adrenoceptors in a similar fashion to β -adrenoceptors through the restoration of receptor numbers and reversal of G-protein- α -adrenoceptor uncoupling. Such a mechanism would explain the upward parallel shift of the PNIF DRC after treatment with fluticasone.

In conclusion, the present study shows that oxymetazoline induced tachyphylaxis of response and associated rebound congestion is reversed by fluticasone.

Tachyphylaxis occurs predominantly at the α_1 receptor type with chronic oxymetazoline dosing with a smaller α_2 component. Further studies are now indicated to evaluate the role of nasal blood flow as an outcome measure in CRS. Research studies would also be welcome in assessing if combination nasal sprays of decongestant and corticosteroid are an effective strategy to obviate tachyphylaxis and rebound congestion in patients with chronic rhinosinusitis.

Chapter 7: Comparative evaluation
of nasal blood flow and airflow in
the decongestant response to
oxymetazoline

Introduction

Nasal blood flow plays a crucial role in many physiological processes such as filtering and conditioning inspired air, counter-current mechanisms in the control of body temperature, nasal cycle, and in various pathological disease processes such as nasal congestion, mucosal hyperaemia and inflammation in rhinosinusitis or possibly in nasal septal ischaemia and perforation(108, 314). The microvasculature of the nose is unique in the human body in that it has multiple components. It consists of copious superficial fenestrated capillaries that have nutritive functions and help with water evaporation, a system of capacitance venous sinusoids with erectile properties that mediate nasal congestion, and lastly numerous arteriovenous shunts, which are probably involved in the warming of inspired air rapidly and in temperature regulation(108, 314). The anatomic structure of these vascular beds in humans is poorly understood, as are the regulatory mechanisms that exert differential influence neurologically or via mediators. We have demonstrated in a previously published study that there is a disconnect between the regulatory mechanisms of venous capacitance vessels and the more superficial blood flow measured by laser Doppler flowmetry(315). We showed that the predominant adrenoceptor type in the venous sinusoids was α -1, the stimulation of which changed the nasal luminal volume and consequently nasal airflow, whilst the blood vessels interrogated by laser Doppler flowmetry was likely α -2. To our knowledge, there is only one study in the literature from 1989 that has evaluated nasal blood flow and its relationship with rhinomanometry in response to a topical decongestant and did not find a significant correlation(316). This is unsurprising as the superficial and deep vascular systems are linked in parallel not in series and have differential regulation and functions,

possibly to preserve mucosal flow during varying levels of nasal congestion(108, 109, 315). What has not been evaluated in the literature is a broad comparative evaluation of nasal blood flow, airflow and subjective outcomes.

We conducted a study to measure parameters of nasal airway patency, nasal blood flow and subjective and objective measures of decongestion using dose response curves, and assessed their reproducibility and responsiveness to decongestion.

Methods

PARTICIPANTS

Using our research volunteer database in Tayside, Scotland, we recruited healthy adults, none of whom were smokers. Inclusion criteria were: male or female healthy volunteers, aged 18 to 65 years, without nasal or ocular symptoms suggestive of rhinitis or nasal polyposis as per the 2008 ARIA(2) and 2007 EPOS guidelines respectively(1), negative skin prick test to common aeroallergens, peak nasal inspiratory flow rate (PNIF) $> 100 \text{ L}\cdot\text{min}^{-1}$ (best effort of three), and PNIF responsiveness to oxymetazoline 0.05% w/v 2 squirts in each nostril (20 min reading) $> 20 \text{ L}\cdot\text{min}^{-1}$. Exclusion criteria were: use of any nasal or oral decongestant, recent or concomitant upper respiratory tract infection, mechanical nasal airway obstruction due to septal deviation greater than 50% at nasoendoscopy, pregnancy, lactation or any medical condition or screening blood result likely to compromise participant safety. The Tayside committee for medical research ethics gave institutional approval to the study and written informed consent was obtained from each participant.

STUDY DESIGN

All authors had full access to the data and manuscript content and contributed to the data analysis. The data for this study were obtained from the screening for a clinical trial, which has been registered in accordance with ICMJE standards at ClinicalTrials.gov (NCT 00487032).

Ours was a prospective observational study in which participants attended the research unit twice at least 48 hours apart. The initial visit determined the inclusion

and exclusion status. A medical history was taken and ear nose and throat examination was carried out. Routine blood tests (full blood count, urea and electrolytes and liver function tests), rigid nasal endoscopy (2.7 mm, 30° Karl Storz-Endoskope; Tuttlingen, Germany) and skin prick testing were performed.

MEASUREMENTS

Each visit started between 8 am and 9 am and measurements were taken at a room temperature of 21–23°C and at constant relative humidity after a 20-minute acclimatization period. No caffeine containing drinks were permitted the morning of the visit and no alcohol for 24 hours. Nasal blood flow was measured using single point laser Doppler flowmetry (LDF) with a custom built PF-5 needle probe attached to the MBF3D™ monitoring system (Moor Instruments, UK). The probe was positioned gently on the nasal mucosa of the inferior turbinate (the position marked and used at each subsequent visit) using a 2.7 mm 30° endoscope (Karl Storz-Endoskope; Tuttlingen, Germany). Horizontal head position and immobilisation was achieved with a custom-made head strap and chin-stabilizer. A micromanipulator with an *x-y-z* axis translator was used for fine adjustment of probe position. An average of 5 minutes of reading was taken after an initial stable tracing of 3 minutes was achieved. PNIF and the total nasal airway resistance (NAR) with active anterior rhinomanometry were used as measures of nasal airways patency. The PNIF measurements were taken as the best of 3 measures from an In-check flow meter (Clement Clarke International Ltd, Harlow, England). Technique was evaluated to ensure a seated posture, horizontal positioning of the meter, correct restoration of the reading to zero, a closed mouth, and an adequate mask seal while making a maximal nasal inspiration. NAR using active anterior rhinomanometry was measured at 150 Pa using an NR6 rhinomanometer (GM instruments, Kilwinning, UK) according to

the recommendations of the Standardisation Committee on Objective Assessment of the Nasal Airway, I.R.S. and E.R.S.(221)

Following the baseline measurements, a dose-response curve was constructed using doubling doses (sum of both nostrils) of oxymetazoline of 25µg, 50µg, 100µg and 200µg at 20-minute intervals. PNIF and NAR were measured at baseline and after each successive dose. Nasal blood flow was measured at baseline, 50 µg and 200 µg. Finally, as a subjective measure of decongestion, participants filled in a decongestion visual analogue scale (0-100 mm) with the minimum anchor '0' denoting no change and the maximum anchor '100' denoting 'most excellent change' after the final dose of oxymetazoline.

STATISTICAL ANALYSIS

The study was powered at > 80 % with an alpha-error of 0.05 (two-tailed) in order to detect a 10L.min⁻¹ difference in PNIF after 200 µg of intranasal oxymetazoline with an estimated sample size of 16 participants assuming the within-subject SD to be 9.2 L.min⁻¹.(205) This was considered adequate to demonstrate a true response to oxymetazoline i.e. change in PNIF within each subject, with adequate consideration for previous literature(298, 305). The distribution of each outcome was assessed for plausibility of the assumption of approximate normality using the Shapiro-Wilk test and by visual inspection of histograms and Q-Q plots, with consideration of previous datasets and literature. The area under the curve (AUC) for the decongestive response to oxymetazoline were calculated at each of the above mentioned time points from baseline using a linear trapezoidal rule. Comparisons for outcomes before and after decongestion were made using a paired t-test with a 2-tailed α -error set at 0.05. Standardized response means were calculated by dividing the mean change in an outcome by the standard deviation of the difference, i.e. a measure of

effect size or responsiveness to provide an indication of the ‘signal-to-noise’ ratio.

All analyses were performed on a per-protocol basis using SPSS version 17,

Chicago, IL, USA.

Results

27 participants were screened of which 21 were eligible for the study according to our inclusion/exclusion criteria. 2 did not fulfil the requirements of the protocol with regard to PNIF technique. 12 female and 7 male participants with a mean (range) age of 33 (21-55) years completed the protocol. There were no serious adverse events.

There were no missing data.

For all outcomes, there were no significant differences between the first and second visits for the baseline and post-decongestion subjective and objective measurements (Table 12).

Table 13: Within subject change in outcomes.

Variable	Visit 1	Visit 2	Mean difference (95% CI; P)	CV (%)
Baseline				
Nasal Blood Flow (units)	277.9 (12.7)	255.7 (13.4)	22.2 (−9.0 to 53.5; .32)	7.7
Nasal Airway Resistance (Pa.s.cm ^{−3})	0.24 (0.03)	0.26 (0.02)	−0.01 (−0.07 to 0.04; .65)	19.7
Peak Nasal Inspiratory Flow (L.min ^{−1})	174.4 (9.5)	178.3 (10.4)	−3.9 (−30.8 to 23.0; .39)	8
Post-decongestion*				
Nasal Blood Flow (units)	138.3 (13)	156.3 (13.5)	−17.9 (−49.2 to 13.3; .78)	12
Nasal Airway Resistance (Pa.s.cm ^{−3})	0.16 (0.01)	0.17 (0.01)	−0.02 (−0.08 to 0.04; .38)	18
Peak Nasal Inspiratory Flow (L.min ^{−1})	223.3 (10.8)	217.2 (12.6)	6.1 (−20.8 to 33.0; .22)	6
Decongestion Dose Response				
AUC Nasal Blood Flow (units.h)	214.5 (13.8)	230.1 (12.8)	−11.5 (−52.1 to 29.1; .56)	12
AUC Nasal Airway Resistance (Pa.s.cm ^{−3} .h)	0.22 (0.03)	0.19 (0.01)	0.03 (−0.01 to 0.07; .13)	15
AUC Peak Nasal Inspiratory Flow (L.min ^{−1} .h)	218.0 (15.4)	207.5 (16.3)	10.5 (7.4 to 28.5; .23)	7
Decongestion Visual Analogue Scale (0 – 100 mm)	37.3 (3.8)	35.5 (4.8)	1.8 (−6.1 to 9.6; .63)	16

All data are arithmetic means (SEM) unless indicated.

*Post-decongestion values given here were measured after the final dose of 200 mcg of oxymetazoline. Visual Analogue Scale scoring was performed after decongestion only.

Abbreviations: CV, within-subject coefficient of variation; AUC, area under the dose response curve.

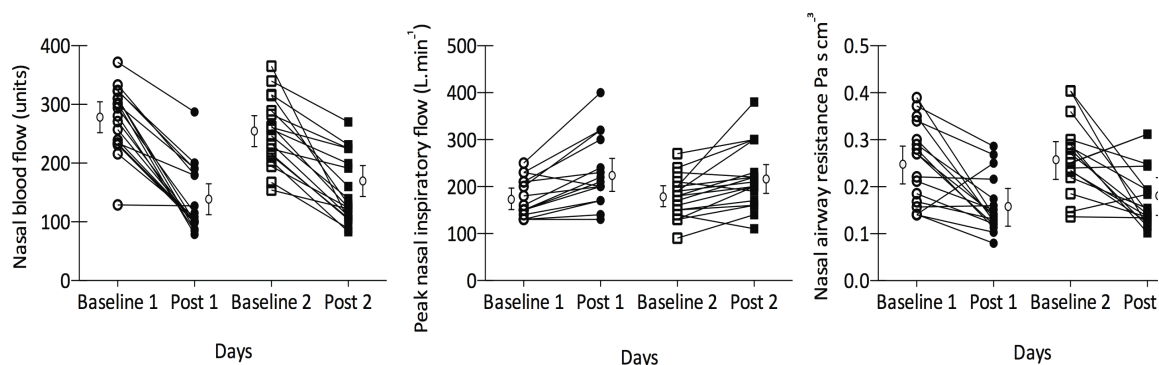


Figure 20: Nasal air-flow and blood-flow outcomes pre and post decongestion.

Decongestive response in nasal blood flow, peak nasal inspiratory flow and nasal airway resistance to 200 mcg of oxymetazoline. Symbols demonstrate individual values. Error bars indicate mean (95% CI). Post decongestion measurements were obtained after the final dose (200mcg cumulative) of oxymetazoline.

After the final dose of oxymetazoline (cumulative dose 200 μg) nasal blood flow decreased by a mean (95% CI, P) of 139.6 (108.3 to 170.8, $P < .001$) units and 99.4 (68.1 to 130.7, $P < .001$) units at the first and second visits respectively (Table 12, Figure 17). Similarly, PNIF increased by 48.9 (22.0 to 75.8, $P < .001$) $\text{L}\cdot\text{min}^{-1}$ and 38.9 (12.0 to 65.8, $P = .003$) $\text{L}\cdot\text{min}^{-1}$, and NAR decreased by 0.1 (0.02 to 0.15, $P < .001$) $\text{Pa}\cdot\text{s}\cdot\text{cm}^{-3}$ and 0.09 (0.02 to 0.15, $P = .002$) $\text{Pa}\cdot\text{s}\cdot\text{cm}^{-3}$ at the first and second visits respectively (Table 12, Figure 21). The area under the decongestant response curve was estimated and was not significantly different between visits for each variable (Table 12). The standardized response means (signal to noise ratio) were estimated for the overall decongestant response for each outcome as follows: NBF, 1.41; PNIF, 1.03; and NAR, 0.97.

The within-subject coefficient of variation was estimated as a measure of reproducibility and the results are given in Table 12. There was no significant

correlation between nasal blood flow, measures of nasal patency and subjective scores (Table 13).

Table 14: Correlation matrix.

Variables	NBF	PNIF	NAR	VAS
NBF	-	-0.18 (.50)	0.01 (.96)	0.04 (.89)
PNIF	-0.18 (.50)	-	-0.33 (.20)	-0.04 (.87)
NAR	0.01 (.96)	-0.33 (.20)	-	0.22 (.40)
VAS	0.04 (.89)	-0.04 (.87)	0.22 (.40)	-

* Values are given as the correlation coefficient R (P value).

Abbreviations: NBF, nasal blood flow (units); PNIF, Peak Nasal Inspiratory Flow ($\text{L}\cdot\text{min}^{-1}$);

NAR, Nasal Airway Resistance ($\text{Pa}\cdot\text{s}\cdot\text{cm}^{-3}$); VAS, Decongestion Visual Analogue Scale (0 – 100 mm).

Discussion

In this study we have demonstrated that nasal blood flow measured by laser Doppler flowmetry is a reproducible and responsive measure that fluctuates in parallel with measures of nasal patency and symptoms.

The first reported use of laser Doppler flowmetry was by Stern in 1975(317). Since then it has been widely applied in various fields other than rhinology. Some of the challenges faced in rhinology are the lack of specific nasal probes, stunted evolution of equipment, motion artefact, the need for a stable head assembly and patient discomfort with the inability to tolerate measurements for long. Consequently, the role of nasal blood flow as an outcome measure within modern rhinological research is yet to be fully determined. This is despite the fact that nasal blood flow is the critical factor underpinning many physiological and pathological processes and is influenced by drugs such as decongestants and corticosteroids among others(318). Laser Doppler flowmetry represents the only non-invasive method currently available for the evaluation of superficial nasal blood flow, while nasal patency measures such as PNIF are presumed as surrogates of venous sinusoidal flow and hence mucosal congestion .

The within subject coefficient of variation for NBF was 7.7 % as compared to 19.7 % for NAR and 8% for PNIF. This shows that NBF measured by laser Doppler flowmetry is reproducible with low variability. PNIF has been shown to be more sensitive and reproducible than acoustic rhinometry or rhinomanometry in evaluating nasal congestion(204, 205). It is also less prone to be influenced by the nasal cycle, the commonest cause of false positive responses in nasal patency testing(101).

In our study, we did not evaluate the effect of the nasal cycle on NBF measured by laser Doppler flowmetry. One reason for this was that decongestant agents abolish the cycle(319). Moreover, the definition of what constitutes a nasal cycle is under debate, quantitative studies having failed to adequately demonstrate the presence of a cycle of spontaneous congestion and decongestion of the venous sinusoids(320). Lastly, while the nasal cycle is purported to play a role in nasal defence, however, its functional significance remains uncertain(314). To our knowledge the nasal cycle has only ever been evaluated in terms of nasal airway patency in the literature(320), future physiological studies would be required to shed more light on this phenomenon. In a recent paper published by our group, we demonstrated a disconnect between nasal blood flow measured by laser Doppler flowmetry and nasal airflow (PNIF, NAR), with regard to response to an alpha-1 antagonist prazosin(315). One plausible hypothesis could be that the regulation of nasal mucosal perfusion is independent of the filling of deep sinusoids, which determines congestion. This would ensure that the nasal mucosal blood flow is kept at near maximum with minimal variation with the nasal cycle.

Standardized response means (SRM) are highly informative measures calculated by dividing the mean change in an outcome by the standard deviation of the difference, i.e. it is a measure of effect size or responsiveness. Because the denominator examines response variance, it provides a sensitive indication of 'signal-to-noise' ratio. It is readily apparent that the SRM of laser Doppler flowmetry was high at 1.41, making it an extremely sensitive indicator of nasal vasoconstriction. Further research is needed to evaluate the utility of nasal blood flow in rhinological research and clinical practice. For example it could prove beneficial in studies looking at adrenoreceptor function, rhinitis medicamentosa, post-operative monitoring of nasal

blood flow or in furthering our understanding of the regulation of nasal function. It could also be useful in the evaluation of intermittent and persistent allergic rhinitis in the research and perhaps in the clinical setting. It could be used diagnostically such as in nasal provocation testing using histamine(321) or adenosine monophosphate(255, 322) and to assess therapeutic efficacy of say intranasal steroids or immunotherapy. Our study has its limitations as an observational study, a placebo-controlled study would be more informative and could be considered in the future.

In conclusion, nasal blood flow using laser Doppler flowmetry is a sensitive and reproducible outcome to decongestion with oxymetazoline, similar to nasal patency and symptoms.

Summary

The current series of studies have explored various treatment paradigms in patients with CRS in order to optimize their therapeutic index and in turn their disease outcomes and quality of life. In this regard, this thesis is unique in the rhinology literature in its balance and bringing together the facets of efficacy and safety, anatomy and physiology, and the macroscopic and the molecular. We believe that one of the strongest points of this oeuvre is in its methodological meticulousness, with the utilization of well-powered outcomes with minimal important differences and in the use of prospective randomized placebo controlled study designs. Finally, while these works hopefully strengthen the body of evidence in rhinology, adding to it, enhancing some aspects; I hope that this also challenges the status quo in a few ways in terms of current guidelines.

The third chapter explores the issue of having a suitable, cheap and portable but sensitive marker of ostiomeatal complex patency in CRSwNP. The importance of the ostiomeatal complex in the disease perpetuation and recurrence is well known in the realm of surgery and treatments are focused on improving paranasal sinus drainage rather than merely debulking nasal polyposis(1). However, nasal endoscopy and CT scanning has its limitations in the assessment of ostiomeatal complex patency. They both provide vital information, but merely demonstrating middle meatal polyps or mucosal thickening on sinus CT scans does not necessarily corroborate with functional OM complex obstruction(12, 42). However, a failed outcome measure in nasal NO may provide the answer. The role of exhaled tidal NO in asthma is well established and it represents a powerful noninvasive biomarker of lower airways

inflammation(223, 323). Unfortunately its counterpart in the nose, the nasal NO has failed to demonstrate similar responsiveness to treatment(230, 255). I believe this is because nasal nitric oxide actually is ‘sinus’ nitric oxide, i.e. the supersaturated reservoirs of NO are actually in the sinuses and these concentrations eclipse the lesser contribution from the nasal passageways. The paranasal sinuses increase the mucosal surface area of the upper airways considerably in comparison with the nasal passage. Indeed, one only has to look at patients with mucociliary dyskinesia and their ‘nasal’ NO concentrations or patients with CRSwNP to know this fact(227, 250, 324). Thus within an inadequate measure for nasal mucosal inflammation resides a hidden gem, which may provide a more true measure of intra-sinus inflammation. Clearly, sinus washouts are not practicable and not likely to be acceptable within clinical practice. While the humming technique requires training and is not intuitive, it is more likely to have a high signal to noise ratio given its supersaturated concentrations in the sinuses(325). We have demonstrated a high inverse correlation ($R = 0.57$, $P < .001$ between humming ‘nasal’ NO and CT scan assessments of OM complex patency(228). Humming NO has also now in this thesis, been proven to reduce substantially with oral corticosteroid therapy and further research is needed to assess it again in AR where previously the results have been disappointing. An approach that uses humming and quiet exhaled NO using a handheld analyzer as described in Chapter 3, could help evaluate post-humming spent NO which is likely a more true reflection of nasal NO levels. Interestingly, we also showed that humming NO correlated significantly only with hyposmia VAS ($R = 0.59$, $P = 0.04$) of all outcomes. It is interesting that humming NO improves in parallel with one of the most disabling symptoms of CRSwNP i.e. the loss of sense of smell. Further controlled studies may be able to differentiate humming responders

and non-responders and possibly see if an improvement in the sense of smell can be predicted based on baseline humming NO or a change in NO after the initial oral steroid bolus. It is also readily apparent that humming nasal NO is the most responsive followed by exhalation and lastly aspiration with the highest SRMs. This will hopefully foster further research in this arena and future guidelines will include this method of measuring upper airways inflammation.

We demonstrate subsequently in Chapter 4, in the first long-term randomized clinical trial looking at sequential induction and maintenance treatment in patients with CRSwNP, the benefits of such an approach on a wide range of subjective, objective and quality measures. Uniquely, this is the first study to also look at a comprehensive array of safety outcomes in the medium-long term. We evaluated stimulated and unstimulated markers of adrenal suppression and bone turnover, to demonstrate clear evidence of safety over 6 months on maintenance topical corticosteroids. Whereas previous studies in healthy volunteers with fluticasone nasal drops and spray have shown low systemic bioavailability even at supranormal dosages, this cannot be generalised to patients with CRSwNP(154). The pan-mucosal inflammation and impaired mucociliary clearance characteristic of CRSwNP may influence nasal retention, corticosteroid absorption and systemic bioavailability, and the use of concomitant inhaled corticosteroid may add to the systemic steroid burden. Notably, improvement in the total nasal symptoms score (TNSS) demonstrated some attrition of effect after switching to nasal sprays. Compared to nasal sprays, drops provide better deposition to the ostiomeatal complex with a low systemic bioavailability(154, 277). However, they are relatively expensive and not universally available. Still this reflects our clinical experience

where carefully instilled nasal drops with the right technique, are more effective than nasal spray.

Interestingly, levels of *staphylococcus aureus* enterotoxin-specific IgE did not change ($P > 0.05$) even with oral prednisolone. We believe that our study was powered adequately to assess this, as evidenced by the marked significant reduction in serum EDN and HS-CRP at 2 weeks. Thus it may be hypothesized that it is the local inflammatory pathways involving the staphylococcal superantigens that play a key role in the amplification and perpetuation of CRS. This is similar to what Van Zele and colleagues have previously reported with a marked increase in local immunoglobulin production with *S aureus* enterotoxin stimulation but not in the serum(49). Future studies could look at these local immunological outcomes with greater effect, using a simple scoop-curette or a polyurethane foam sampling technique developed by Deutschle and coworkers, to make it more acceptable for patients and to evaluate a variety of mediators at once(220, 326).

Our findings in Chapter 5 question the the EAACI/GA2LEN 2007 guidelines in recommending routine aspirin challenge testing in CRS. In our experience of treating patients with chronic rhinosinusitis and asthma for over a decade, we have most commonly seen clinicians rely on a history of aspirin sensitivity rather than offer a provocation test in a general group of CRS patients. While this may be said to underdiagnose patients with ‘aspirin sensitivity’ in that there are likely to be undiagnosed patients who may have a positive challenge test, routine testing in an unselected group of patients with CRS may be of very low yield. Our results do not support this recommendation and further research is needed to identify the phenotype/endotype of CRS most likely to benefit from this approach. I hope these

results will inform further evaluation of steroid-refractory AIR patients in terms of aspirin desensitization therapy, which I believe is an underutilized tool.

Perhaps the findings in Chapters 6 and 7 are of greatest importance in creating new uses of existing medical therapy in CRS/AR, in terms of increasing the time for which nasal sympathomimetics can be safely prescribed in these patients by modulating adrenoceptor tachyphylaxis with the concomitant use of corticosteroid. This is potentially of tremendous benefit in acute exacerbations of rhinosinusitis, where there is a need for a fast-acting efficacious and safe decongestant. Further studies, could evaluate a combination spray of corticosteroid and sympathomimetic in AR/CRS. Moreover, it can lead to more molecular level studies where receptor upregulation studies in the human nose can lead to a new area of research into the little understood regulatory system of the human nose.

While it is well known that topical sympathomimetics are highly efficacious decongestants, the role of the alpha-adrenoceptor and the cross-talk with nasal inflammation may open up new avenues of research. Since the work of Hanania and coworkers has opened a new and paradoxical view of traditional pharmacology with their open label study in 10 subjects with asthma, in which nine weeks of nadolol treatment produced a significant, dose-dependent increase two-fold increase in the methacholine PC20 at 40 mg ($p < 0.0042$)(327). However, there was also a minor dose-independent 5% reduction in mean FEV1 over the study period ($p < 0.01$). In our current experience, this is still an area of promise despite negative early results in the first randomized clinical trial in asthma using propranolol(136). Now there is evidence that prazosin acts as an inverse agonist at the alpha-1 adrenoceptor in chronic dosing regimes(328, 329). This area holds tremendous potential in rhinology in terms of if there is an inverse agonist effect of prazosin in the human nose, this

may provide a new inexpensive treatment for nasal congestion and inflammation by altering nasal hyper-reactivity similar to the findings by Hanania et al(327).

The collection and reporting of patient reported and disease-sensitive and specific outcomes is a key priority as set out in the Government's July 2010 White Paper, *Equity and excellence: Liberating the NHS* and *The NHS Outcomes Framework 2012/13*. Indeed, rhinology is a specialty that has traditionally lagged behind in having validated, reproducible disease-specific outcomes, although considerable and commendable progress has been made in the last decade(1, 6). Such a need also exists in the realm of rhinology research where intervention-sensitive, precise and accurate outcomes are evolving currently. Rhinology belongs to a group of traditionally undervalued illnesses in terms of recognition, trivialization of symptoms, adequate therapy, poor research funding and lack of national awareness of its tremendous impact on the youth, the productive and the able. The research conducted by the Asthma and Allergy Research Group along with their clinical partners in Otorhinolaryngology, the basic sciences, and family practitioners represents a multidisciplinary approach to address a much neglected but incredibly vital area of research and clinical need.

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